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A. D. MELVIN, CHIEF OF BUREAU.

A COMPARATIVE STUDY OF TUBERCLE
BACILLI FROM VARIED SOURCES.

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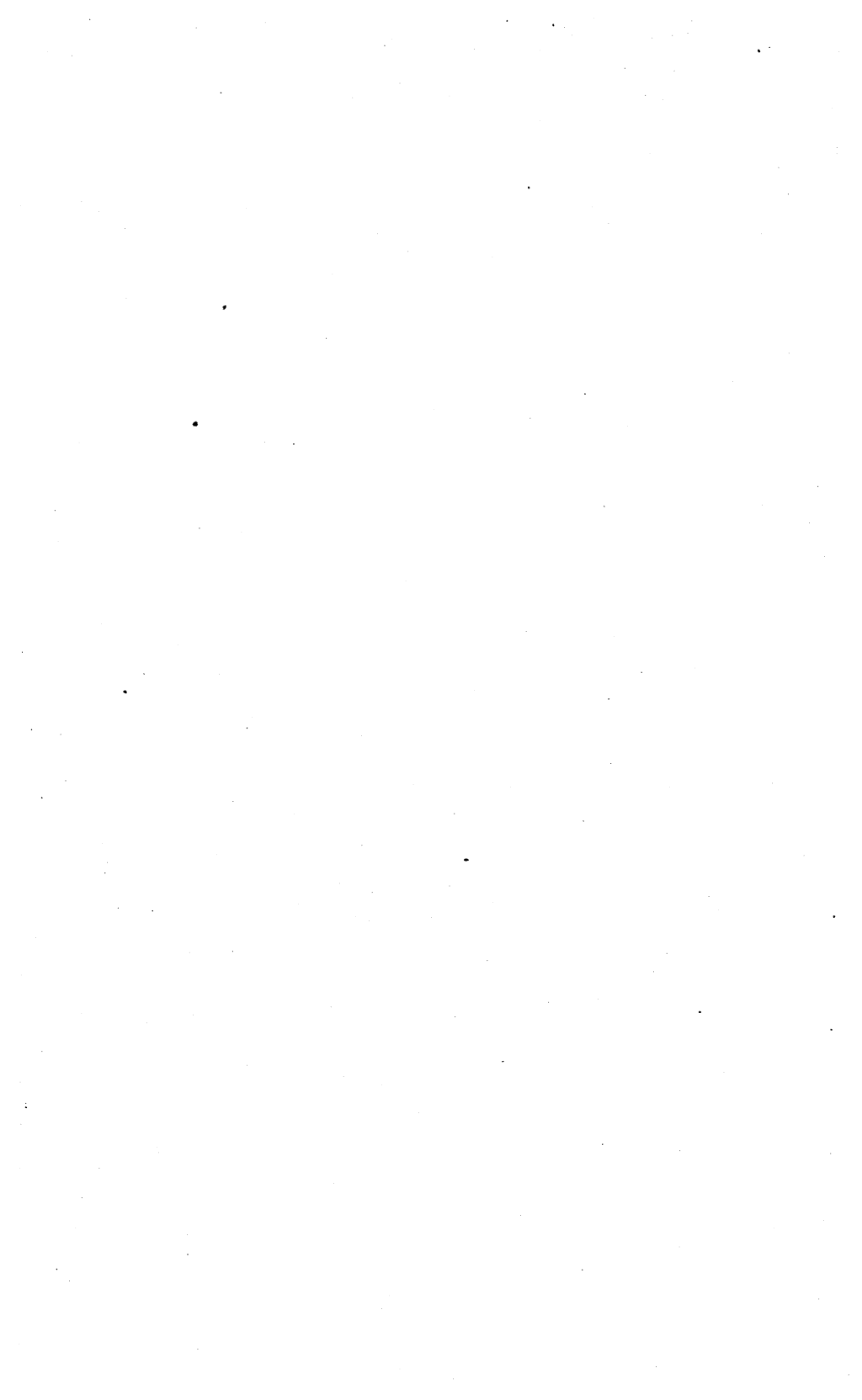
SIR: I have the honor to transmit herewith a paper entitled "A Comparative Study of Tubercle Bacilli from Varied Sources," by Drs. John R. Mohler and Henry J. Washburn, of the Pathological Division of this Bureau, and to recommend that the same be published as Bulletin No. 96 in the series of this Bureau.

This work comprises a series of experiments upon several species of animals with the main object of shedding further light upon the question of the intertransmissibility of the human and bovine types of tubercle bacilli. The investigation has brought out what is regarded by the authors as strong additional evidence for the affirmative. It is unnecessary, therefore, to point out its bearing upon the fight which is being waged against tuberculosis and the importance of excluding from human consumption the meat and milk of tuberculous animals.

Respectfully,

A. D. MELVIN,
Chief of Bureau.

HON. JAMES WILSON,
Secretary of Agriculture.



CONTENTS.

	Page.
Introduction	7
Object of the investigation.....	8
History of similar investigations.....	9
Origin of cultures used in present investigation.....	20
The cultures selected for the tests.....	25
Technique of the experiments.....	26
Isolation of cultures.....	26
Preparation of suspensions for inoculations.....	27
Dosage.....	28
Staining of bacilli.....	29
Appearance of cultures on dog serum.....	29
Characteristics in subcultures.....	33
Morphological examinations	33
Review of morphological results.....	36
Pathogenesis.....	38
Virulence for rabbits.....	38
Results of rabbit inoculations.....	39
Summary of rabbit inoculations.....	44
Virulence for white mice.....	46
Virulence for cats.....	47
Results of cat inoculations.....	47
Summary of cat inoculations.....	50
Virulence for dogs.....	53
Results of dog inoculations.....	54
Summary of dog inoculations.....	56
Virulence for goats.....	58
Results of goat inoculations.....	58
Summary of goat inoculations.....	60
Virulence for sheep.....	60
Results of sheep inoculations.....	61
Summary of sheep inoculations.....	63
Virulence for cattle.....	64
Results of cattle inoculations.....	65
Summary of cattle inoculations.....	73
Histologic examination of lesions produced.....	74
Chemical reaction of glycerin-bouillon cultures.....	78
Conclusions.....	82
Bibliography.....	85

ILLUSTRATIONS.

	Page.
PLATE I. Various forms of human tubercle bacilli.....	32
II. Various forms of human and bovine tubercle bacilli.....	32
III. Various forms of bovine tubercle bacilli.....	32
IV. Lung of sheep No. 104, inoculated subcutaneously with human tuberculosis (Boy V)	64
V. Lung of heifer No. 302, inoculated subcutaneously with human tuberculosis (Boy V)	64
VI. Miliary tuberculosis affecting the lung of heifer No. 297, inoc- ulated subcutaneously with human tuberculosis (BB)	64

A COMPARATIVE STUDY OF TUBERCLE BACILLI FROM VARIED SOURCES.

INTRODUCTION.

For many years tuberculosis has been regarded as one of the most serious affections common to man and animals. Because of the similarity of the microscopic lesions produced by this disease in all species of the domestic animals, as well as in man, it was formerly considered that, wherever found, the affection was sufficiently described by the simple name of tuberculosis. With the announcement of the discovery of the specific micro-organism of tuberculosis by Robert Koch, in 1882, there was awakened a new interest in the study of the disease, and as a result of this (from a bacteriologic view point) classic investigation of the affection in its various phases, it was accepted on all sides as a fact that tuberculosis, whether found in man, bovine, or bird, owed its origin in every instance to one and the same species of bacillus. Aided soon afterwards by Ehrlich's discovery of the peculiar staining characteristics of the organism, nothing to prove the identity of the slender bacillus obtained from tubercular lesions in various species of animals seemed lacking.

In 1896 Theobald Smith^{30 a} called attention to the existence of certain differences between tubercle bacilli obtained from cattle and those presumably from man, which he confirmed in 1898, but the subject failed to arouse any universal comment until Koch, in 1901, after repeating in substance the statement made by Smith, continued by expressing the opinion that there was but little danger of man ever becoming infected with tubercle bacilli of bovine origin, because of the fact that bacilli of human and of bovine sources were different and were not transmissible between human and bovine subjects.

The amount of research work which has since been performed by investigators in their attempts to answer some of the numerous questions which at once arose upon the admission of material differences between tubercle bacilli of varied origin is truly amazing. Medical literature has been flooded with expressions of opinion pro

^a The figure references refer to bibliography at end of bulletin.

and con, and with reports of experiments made for the purpose of gaining further light on a vexatious question. Many valuable deductions have been obtained and much that is instructive has been achieved through these efforts.

OBJECT OF THE INVESTIGATION.

The work of which this article is a record was undertaken for the purpose of repeating the experiments performed by Smith, and of confirming or disproving the subsequent investigations of Koch, who, while in a general way verifying the results obtained by Smith, went much further in drawing his conclusions, asserting not only that there is a distinct difference between human and bovine tubercle bacilli, but that cattle can not be infected with the human bacillus and that man is not susceptible to the bovine organism, except on the rarest occasions. While Smith's conclusions were based upon an elaborate, well planned, and scientifically executed research, which included not only a full description of the morphology and cultural characteristics of the human and the bovine tubercle bacillus, but also parallel inoculations of five species of animals, Koch's assertions were apparently the result of a comparison of only the pathogenic effects of these bacilli on two species of animals.

The demands made by Smith for the repetition by other investigators of the work performed by him can be met, but the demands first made by Koch are only practicable in so far as they relate to the inoculation of cattle with bacilli of human origin, since direct proof that would be forthcoming by inoculating man with the bovine bacillus can not be obtained. And the later address by Koch in 1902 also postulates conditions incapable of proof. It has therefore been our endeavor to follow out as closely as possible the earlier investigations of Smith, which include the only practical experiments demanded by Koch—the inoculation of cattle with bacilli from man—stated by the latter to be the only test necessary to discriminate between human and bovine bacilli.

The practical importance of having exact knowledge of the intertransmissibility of human and bovine tuberculosis has long been realized by the sanitarian and was immediately recognized by the public when it became acquainted with the statements made by Koch. As a result of Koch's opinions health officers in the enforcement of their regulations at once met with opposition from the people who are always willing to grasp at a new thought or idea. But the results obtained by the majority of those who immediately undertook to solve this problem were in opposition to Koch, and they almost unanimously recommended that it would be unsafe to relinquish or modify the present sanitary police regulations with reference to bovine tuberculosis.

While our work has been carried out along the above-mentioned lines, other methods of experimentation suggested themselves and were inaugurated, but can not find a place in this paper. The objections that have been frequently raised to results obtained with old cultures, also by the injection of large doses and by certain methods of inoculation, have been met by working in all cases with freshly isolated bacilli from natural cases, by giving small, uniform, and well-proportioned doses, and by using the subcutaneous method of inoculation in all cases except in a number of cats, which received intrathoracic injections.

HISTORY OF SIMILAR INVESTIGATIONS.

As early as 1896 Theobald Smith³⁰ called attention to certain differences in morphology, biology, and pathogenesis which he had found to exist between bovine tubercle bacilli and a culture presumably of human origin. This was followed in 1898 by a more extensive work,³¹ which later became world renowned because of the freshly discovered facts which it brought forward. It was here shown that tubercle bacilli of the bovine type are short, straight, not very regular in outline, grow less vigorously upon artificial media than sputum bacilli, are much less influenced by certain modifications of the culture medium, and, finally, tend to remain short when grown artificially through several generations, while the sputum bacilli grow profusely and quickly become long and slender during cultivation, if not so at the start.

A marked difference in pathogenic power was also shown between tubercle bacilli of the bovine type and those from sputum. In this experiment of Smith's the cultures of human tubercle bacilli were all obtained from sputum and no attention was given to cultures from tuberculous tissues.

Despite the merit and vast importance of this subject, very little attention was given these results by sanitarians until the appearance of the very radical statements of Koch in 1901. He, in collaboration with Schütz,¹⁵ reached the conclusion, after a series of comparative tests by means of subcutaneous, intravenous, and intraperitoneal inoculations, as well as by feeding and inhalation tests, that cattle are not susceptible to bacilli obtained from human tuberculosis, while bacilli of bovine source invariably attack them with great rapidity and virulence. Reasoning from these discoveries, they advanced the opinion that bovine and human tuberculosis were sufficiently different to preclude their intertransmissibility from one of these hosts to the other. They also suggested that one could readily determine by inoculating certain tubercle bacilli beneath the skin of cattle whether they were derived from a human or a bovine subject. Immediately following these statements, it was very nat-

ural that numerous investigators should direct their energies toward the settlement of the contested point—that members of the human family were not susceptible to bacilli of bovine origin, and, conversely, that bovidæ could not be successfully inoculated with tubercle bacilli from a human source. Since human and bovine bacilli comprise the most important types known, much of the comparative work has been centered around them in previous work, and they will also form the basis of the present article. In the five intervening years many instances have been cited of the infection of man through cuts and injuries received while slaughtering tubercular cattle. Numerous attempts have been made to infect cattle through various methods of inoculation with human sputum, or with cultures derived from human cadavers. The results have been varied, but the conclusion of the majority of the investigators is strikingly uniform.

Even at the time that Koch and Schütz were making their comparative investigations Ravenel^{27,28} was actively employed in similar experiments. His conclusions were very different from those of Koch and Schütz, as will be seen from the following extracts from an address in which his work and its deductions were clearly described:

While it has been shown conclusively that a greater pathogenic power is the most distinguishing characteristic between the bovine and human tubercle bacilli, even this is not constant.

The pathogenic action of the bovine tubercle bacilli for man is seen where a careful study of wound infection is made. A comparison with similar infections with human tuberculous material shows that the bovine bacillus grows well in the human body, under the most unfavorable circumstances producing typical lesions, and that it shows for man at least as great pathogenic power as the human bacillus under identical circumstances. * * * The most direct and positive proof that bovine tuberculosis is responsible for a certain amount of human tuberculosis is given by the finding of the bovine bacillus in lesions of man. In June, 1901, I succeeded in isolating from the mesenteric glands of a child sent to me by Dr. Alfred Hand from the Children's Hospital, in Philadelphia, a culture which has been designated BB. This culture showed the most intense virulence for cattle, killing two calves in seventeen and twenty-seven days, respectively, and a 6-year-old cow in seventeen days. Excepting in the first generation, when some irregularity of staining was noticed, this culture has always shown every characteristic of the bovine tubercle bacillus, and must be regarded as having come directly from cattle. * * * I have since isolated two other cultures from mesenteric glands of children which have unusual virulence.

Culture U produced general infection in a calf with moribund condition in forty-six days, and the other possessed even greater virulence for cattle and was considered bovine by Ravenel.

Vagedes (1898)³⁴ was one of the earliest writers to notice differences in virulence and morphology between various cultures of tubercle bacilli, but his investigations did not serve to place human and bovine tubercle bacilli in separate classes, as he found certain human

cultures which he rated with his bovine cultures in view of their virulence for test animals and in view of their shortness of form when grown artificially.

A test of the comparative virulence of eight cultures of tubercle bacilli which were all derived from human sources was made by Veszprémi (1903)³⁵, with the result that the greatest variation in the degree of virulence was determined. The tests of Veszprémi were based upon the inoculation of rabbits, and he found that no constant standard of virulence can be established for tubercle bacilli from human sources. The avirulent cultures disappeared from the rabbit's organism without leaving behind any trace. Those of slightly greater virulence, when used in sufficiently large quantities, possessed the power of producing tubercles, but no caseation developed, and there appeared to be no increase in the number of tubercle bacilli within the tissues. When tubercle bacilli of a still higher degree of virulence were used, the lesions produced by them were very similar to those caused by the use of tubercle bacilli of slightly lower virulence, but they retained the capability of multiplying within the tissues and probably also of generating a slightly toxic agent. The most virulent cultures produced tubercles in which the tubercle bacilli multiplied rapidly, caseation was widespread, and strong toxic effects were evident.

These tubercle bacilli also possessed a marked capability for spreading about in and overpowering the animal organism into which they were introduced. It is also of interest to notice the differing degrees of susceptibility shown by the rabbits used in this experiment. For instance, two rabbits were inoculated intravenously by Veszprémi with equal amounts of a culture which he designated as culture G. Although both of these test animals developed generalized tuberculosis, one of them died in twenty-eight days as the result of inoculation, while the other one lived for fifty-seven days and was finally killed in order that the extent of its lesions might be determined. As will be mentioned more fully later in this work, this experience of Veszprémi has been often recalled to our minds by the marked and baffling differences of susceptibility shown by animals of similar species, age, and weight for duplicate doses of tubercle bacilli, and indicates the importance of recognizing and accepting the existence of marked differences in individual resistance.

That section of the report of Kossel, Weber, and Heuss¹⁶—the German Tuberculosis Commission—which records the results of their subcutaneous inoculations of cattle with bovine tubercle bacilli may with advantage be taken up for consideration immediately after this article by Veszprémi. In the commission's experiments (1904) ten cattle were used, with the result that five died of generalized tuberculosis after forty, forty-four, fifty-four, fifty-six, and sixty-five days,

respectively. Four were slaughtered after periods ranging from four to seven months. These showed generalized tuberculosis, but the disease was more confined to the lymph glands and had therefore produced a more chronic type of the affection. Finally, the effects of the inoculation of one of the cultures were limited exclusively to the prescapular gland at the site of the inoculation.

Here it will be seen that marked variations may occur in the virulence of tubercle bacilli of bovine origin as well as in cultures derived from human tubercular lesions and sputum. Of the thirty-nine specimens of human tubercle bacilli examined by the commission, four were found virulent for cattle, and those four were all derived from lesions in children.

A review of tests with bovine tubercle bacilli made upon rabbits by Ravenel (1901)²⁷ shows that his bovine culture T3 killed the animals in an average time of forty-nine and one-half days, while bovine culture F6 required one hundred and twenty-six and one-half days on an average for the production of fatal results upon similar animals. That the variations of bovine tubercle bacilli are not limited to virulence is shown by the following statement in the bulletin above quoted:

In a series of examinations of material coughed up by cows I failed always to find bacilli of what has been described as the bovine type. In fact, the longest bacilli I have ever observed, except in old cultures, were seen in some specimens of this material.

Ravenel has also recorded the infection of three cattle by means of intraperitoneal inoculation with human sputum.

His stand upon the question of the unity of human and bovine tuberculosis is expressed in the following unmistakable lines:²⁸

Although at the time of the London Congress on Tuberculosis I had collected a certain amount of evidence which convinced me of the correctness of my belief in the essential unity of tuberculosis as seen in man and in cattle, and in the intercommunicability of the disease between these two species of mammalia, research in every part of the world has been so stimulated since that time that to-day we are in possession of facts that seem to me to be irrefutable. It appears to me that the case might well rest on the positive evidence afforded already by the discovery of the bovine germ in children.

Schottelius (1903)²⁹ has reported positive results obtained through feeding human sputum to cattle. The outcome of these cases was such that the writer came to the conclusion that there are no proper grounds for the belief that cases of tuberculosis of cattle due to the ingestion of human sputum at frequent intervals will always heal rapidly and spontaneously. On the contrary, in each of his experiment cattle he found the lesions so general that they must have speedily had a fatal termination had he not slaughtered the animals for purposes of investigation.

By means of his investigations Prettner (1903)²⁴ has found that typical tubercular lesions follow the inoculation of calves with certain pure cultures of human tubercle bacilli. The surest way of inoculation is intraperitoneal. Cultures of high degree of virulence will here produce acute miliary tuberculosis, in which bacilli are typical, abundant, and virulent.

Mention has been made in previous bulletins of this Bureau of the results obtained by Cippollina, Bang, Orth, Fibiger and Jensen, Karlinski, Thomassen, Arloing, Nocard, Stenstrom, Svensson, Dean and Todd, De Jong, Wolff, De Haan, and Hamilton and Smith, who succeeded in producing well-marked lesions of tuberculosis in cattle with tubercle bacilli derived from man, and in many instances these bacilli were as virulent as those obtained from cattle.

Preisz (1904)²³ found among ten different cultures of human tubercle bacilli none that would produce generalized tuberculosis in cattle. They showed slight variations in virulence, however, as one produced not the slightest lesion, while the most virulent caused an extension of tubercular formation from the point of inoculation to the adjoining lymph glands.

Klebs and Rievel (1902)¹⁴ experienced no difficulty in producing a fatal infection in a calf by causing it to inhale tubercle bacilli derived from a human subject.

The following is quoted from Von Behring (1902):²

In spite of all cultural differences, and in spite of the right material differences in the anatomic appearance of the disease products developed in small and large test animals, we yet have a perfect right to declare that our avian tubercle bacilli and bovine tubercle bacilli are identical species. I mention this because in the following work our original avian culture, and the many modifications of it which we have obtained by its repeated passage through animals, are considered simply deviations of our bovine tubercle bacilli.

I also find that there is less difference between our bovine culture (TB. II) and our human culture (TB. I) than there is between two of our human cultures (TB. I and TB. Ia). I find that there are cultures of tubercle bacilli coming from man that have but very slight pathogenic power for cattle, and others which are as virulent for cattle, or even more virulent, as many cultures of bovine origin. In direct opposition to the teaching that bovine tubercle bacilli are harmless for man, I have every ground for the statement that bovine tubercle bacilli in general represent a higher grade of virulence than the human tubercle bacilli, and are therefore more dangerous for man.

Although Delépine (1902)⁶ has found that human tubercle bacilli are as a rule less virulent than those of bovine origin, he has nevertheless found human tubercle bacilli that were just as virulent as those derived from cattle. He used human sputum in his investigations and produced positive results in cattle in two instances.

Bujwid (1904)³ reports himself positively decided in opposition to both Koch's and Von Behring's theories. By means of his own experiments he has reached the conclusion that there are different

varieties of tubercle bacilli which may reach greater or less degrees of virulence. He found that much depended upon the amount of infectious material which was injected into the system. His tests with cattle proved that they could be injected with human tubercle bacilli, but that the lesions which resulted always showed greater tendency toward localization. He also found that there are differing degrees of virulence possessed by various cultures of avian tuberculosis.

Hueppe (1904)¹² states that tubercle bacilli have developed phylogenetically from saprophytes out of the group to which the actinomyces belong, but he thinks it improbable that any such transformation is occurring at the present time. Their virulence is most varied and is by no means constant even in the same species of animal. The statement that human and bovine tubercle bacilli are entirely different species has been repeatedly disproved. Their differences are only modifications of one and the same species, caused by their adaptation to various kinds of animals. This explanation of variations will also apply to avian tubercle bacilli, they being but forms of the common family. Tubercle bacilli which have become adapted to the tissues of cattle are the most virulent.

Lartigau (1901),¹⁷ starting out with the idea that possibly bacilli derived from different sources might vary considerably in virulence, obtained cultures from various lesions in human beings, such as tuberculous lymph glands, pulmonary tuberculosis, and bone and joint tuberculosis. He found that the morphologic features were fairly uniform. In those organisms in which the virulence was greatest the growth upon culture media was usually least. In one instance a bacillus was obtained from a human being that produced a culture not unlike that described by Theobald Smith as characteristic of bovine tubercle bacilli. This bacillus was excessively virulent and corresponded also in this respect with Smith's description of the bovine bacillus. The inoculation experiments confirm Lartigau's belief that the virulence of the tubercle bacillus varies considerably.

The culture referred to by De Jong¹³ in 1902 is worthy of particular attention because of its derivation from sputum; yet notwithstanding this fact it was shown to possess all of the leading characteristics of bovine tubercle bacilli. With this culture he inoculated a calf and a goat intravenously. The calf died nineteen days afterwards with generalized tuberculosis, while the goat succumbed after twelve days, showing tuberculosis of the lungs, liver, and spleen. De Jong concludes from certain of his experiments that while bovine tubercle bacilli are usually more virulent than those of human origin, this is not always the case.

Sturmann (1903)³³ obtained cultures of the above sputum bacilli

isolated by De Jong and by means of continued experiments succeeded in substantiating the statement that they conformed to the bovine type in every important particular.

Among the guinea pigs used in making comparative tests of tuberculins by Wolbach and Ernst (1904)³⁰ was one series consisting of ten, one half of which were inoculated with human tubercle bacilli and the remaining half under identical conditions with bovine. One of the animals receiving the bovine organism far outlived all of the others, although it finally developed generalized tuberculosis, thus indicating either a remarkable natural immunity of the animal or a lower degree of virulence in this bovine bacillus.

In a later series of experiments Smith (1905)³² obtained two fresh cultures of human tubercle bacilli, No. XI and No. XII, which were isolated from mesenteric lymph nodes of children. The bacilli of No. XI, when first recovered, were shown microscopically to be as follows: "A few stained solidly; many appeared granular—fragmented. They were frequently in groups of three or more individuals ranged parallel to one another." After passage through a single guinea pig "the bacilli from all cultures examined were plump rods, many of them thick centrally and tapering somewhat toward both extremities, or slightly thicker at one end and tapering toward the other. They were straight or very slightly curved." Measured, when first recovered, they were found to be $2\ \mu$ or more in length; after passage through animals they were shorter, being 1 to $2\ \mu$. In pathogenesis culture No. XI approaches but does not quite attain the virulence of fresh bovine cultures. Its morphology is that of the bovine race. Culture No. XII, on the other hand, is essentially of the human type both as to morphology and pathogenesis. It is significant that these two quite divergent races of tubercle bacilli were associated with diseases very much alike in the human subject.

He further states:

There is no uncertainty as to the capacity of XI to produce genuine tuberculous lesions in cattle. It is not to be denied that its pathogenic power appeared to be somewhat below that of cultures from cattle; but we must here take into consideration the temporary sojourn of XI in the human subject and the possibility of a slight modification therein.

MacFadyean (1903)¹⁸ used both inoculating and feeding tests upon 18 monkeys, and reached the conclusion that monkeys were susceptible to bovine tubercle bacilli either directly or after one passage through the organism of the guinea pig. Animals used in feeding experiments succumbed to general tuberculosis after a like period of time whether the material fed them originated in human or bovine sources. Thus the young monkey proved to be equally susceptible to the human and the bovine strains of tubercle bacilli. The striking

difference in the effects produced occurred in the digestive tract. Intestinal lesions were found in the case of every monkey that had been fed with human tuberculous material, while none of the monkeys fed with the bovine material presented any evidence of tuberculous ulcers in the intestine. Within the limits of these experiments it would appear that a food tuberculosis can be as readily brought about by the bovine as by the human strain of the tubercle bacilli in the young monkey.

Chiesi (1904)⁴ studied 24 cases of tuberculosis and reached the following conclusions:

The morphology and chemical characteristics of tubercle bacilli vary in different individuals and at times in the same individual. The morphologic variations in the germ are, as a rule, more numerous and more marked when there is mixed infection. Other things being equal, the severest forms of tuberculosis show, for the most part, short and easily stained bacilli, while the lighter forms show bacilli with extensive morphological changes and poor staining qualities. If kept in the thermostat at 37° C. the tubercle bacillus tends to grow and to change the shape. In sputum kept in this manner we find altered forms next to normal forms. Tubercle bacilli kept in sputum where they occurred become attenuated in the course of twenty days and lose their virulence.

After giving a résumé of the cultural and morphological differences between animal and human tubercle bacilli Beck (1903)¹ writes:

Comparative tests of both kinds of tubercle bacilli in regard to their pathogenic effect upon various animals show that in general the bovine tubercle bacilli evince a greater tendency toward a generalized attack upon the animal. The dog alone offers an exception to this rule. With this species of animal intra-abdominal inoculations with human tubercle bacilli resulted in an attack upon the lungs in addition to the infection of the abdominal organs, while the lesions resulting from similar inoculation with cultures of the bovine type were limited to an attack upon the abdominal organs.

The animals inoculated with bovine tubercle bacilli died a little earlier than those treated with human tubercle bacilli, and as the lesions in the former gave no evidence of a tendency to remain stationary, it is possible that they would in time have forced their way into the thoracic cavity.

Moeller (1903)¹⁹ found that tubercle bacilli that would grow artificially from sputum without passage through animals were less virulent than those isolated by means of animal passage. He found a marked difference in the virulence of various cultures of human tubercle bacilli, but thought that he was able to account for this through the action upon them of the tissues of the guinea pigs through which they had been passed.

While exhibiting some tubercular tissues which were produced by the inoculation of calves with human tubercle bacilli of a virulent order, Orth (1903)²⁰ made the following statement:

The preparations are here again to-day, and you can easily see that the tuberculosis has originated at the point of inoculation, and that the lymph

glands in the second calf also, as in the animal directly inoculated, exhibit the same rapid calcification which is so characteristic of *perlsucht*; that is to say, their condition does not resemble that common in human tuberculosis, but genuine *perlsucht*, although the infection was with bacilli derived from man.

Herzog (1903)¹¹ found that in his experiments relative to the survival of mammalian tubercle bacilli, when introduced into the systems of cold-blooded animals, the same positive results were obtained when bovine tubercle bacilli were used instead of those of human origin.

Pansini (1894)²¹ isolated tubercle bacilli having the characteristics of the avian variety from a human being after passing the tuberculous products through guinea pigs. He produced tuberculosis successfully in guinea pigs with avian tubercle bacilli, and says that there can be no doubt of transition forms between the avian and mammalian types.

Phisalix (1903)²² studied the characters of a culture which he derived from a tubercular intestinal lesion in an emu. This developed both the characters of the avian and of the human types in different cultures upon glycerin-potato. Inoculated into the peritoneal cavity of guinea pigs, it caused the death of these animals in fifteen to twenty days. Cultures grew readily from the lesions of this animal, but at once assumed characters which belong to the human type rather than to the avian. Because of its exceptional virulence for the guinea pig, and of its assumption of the characters of the human tubercle bacilli culturally, Phisalix concludes that the culture in question represents a transformation stage and presents characters of both human and avian types.

Ramond and Ravaut (1898)²⁶ inoculated frogs with human, avian, and piscine tubercle bacilli, with the result that the avian cultures proved to be the most virulent. The human and piscine forms showed equal virulence.

Through careful investigations Eberlein (1899)⁸ has established the fact that tuberculosis of the parrot and tuberculosis of mammals are identical, wherefore he considers the former of considerable importance to man. He found that culturally both grew alike, that microscopically both types appeared alike, and that when inoculated into guinea pigs both produced similar generalized lesions of tuberculosis. He readily infected parrots with tubercular material from man, citing five cases.

Friedmann (1903)⁹ gains from his extensive investigations of turtle tuberculosis a few results that are of special interest when applied to the present question of the identity or nonidentity of all types of tubercle bacilli. He used in his work cheesy nodules from the

lungs, and had no trouble in obtaining pure cultures from these upon the commonly used culture media at a temperature of 37° C. They showed remarkable similarity to the human type even in the first cultures, and the similarity was enhanced as the result of reinoculation and transfer to new cultures until at last even the most expert specialists in the cultivation of tubercle bacilli were unable to differentiate between them.

While most of the investigations previously carried out with avian tuberculosis have been limited to tubercle bacilli obtained from hens and parrots, Rabinowitsch (1904)²⁵ was able to work with birds of various kinds and succeeded in isolating 34 different cultures with which to carry out comparative studies. The statement is often made that tuberculosis of the lungs is of very rare occurrence in birds, but out of 55 tuberculous birds this investigator found tuberculosis of the lungs 35 times, and in 5 instances the lesions were limited to an attack of the lungs.

It was also found that the infection of birds did not occur, as had been formerly supposed, only through the ingestion of feces which were contaminated with tubercle bacilli, but that the disease was often spread to birds through the eating of rats or mice which had become spontaneously affected with avian tuberculosis. Weber and Bofinger suggested that these rodents might play an active part in the spread of avian tuberculosis, and Rabinowitsch believes that her experiments have fully proved the fact.

Although avian tubercle bacilli present a peculiar appearance when compared in their manner of growth artificially with that of mammalian tubercle bacilli, Rabinowitsch has found among 34 cultures 2 which grow exactly like mammalian cultures both upon solid and upon liquid media. In addition to these there were a number that approached closely the mammalian type in their manner of growing, and it required continued growing through later generations to prove that they belonged by the majority of their characteristics to the avian type. It has been frequently shown by experiments with bovine and with human tubercle bacilli that there is a great degree of variation of virulence to be found between different cultures derived from tubercular subjects of the same species, and the comparative studies of the avian cultures have proved that such variations of virulence between different cultures of avian tubercle bacilli are also noticeable.

The use of tubercular material direct from birds in the inoculation of guinea pigs gave tuberculosis of equal severity with that resulting from inoculation with mammalian material in several cases, while in other instances the course of the disease was less severe. The use of pure cultures of avian tubercle bacilli for inoculation purposes resulted in a lessening of virulence in the case of tuberculosis

produced. Repeated passage of avian tubercle bacilli through guinea pigs failed to enhance their virulence; in fact, some of them became so attenuated by this means that they would no longer produce tubercular lesions in guinea pigs after the fifth or sixth passage. It was found also that certain fishhawks could, like the parrot, become infected spontaneously with mammalian tuberculosis.

Rabinowitsch concludes from these investigations that exchanges of position between avian and mammalian tubercle bacilli are not so infrequent as has been formerly supposed, and the earlier reports of avian tuberculosis appearing in man should therefore be granted greater credence. The results here gained seem to justify one in claiming that avian and mammalian tubercle bacilli are not distinctly separated kinds of bacilli, but only varieties of a single species that have become differentiated through their retention in the bodies of different species of animals.

At the veterinary high school in Hanover various tests were carried out in 1903 and 1904 for the purpose of verifying the results obtained by the German Tuberculosis Commission at Berlin. In his preliminary report of this work Dammann (1904),⁵ by whom the tests were made, says:

The complete report of the experiments of our institute will not be prepared for a few months yet, but if I select one of our cases and give it out for publication now it is because it strengthens with special force the conviction of the possibility of the transmission of human tuberculosis to animals. The case is worthy of the most careful consideration, because it shows that there are cultures of human tubercle bacilli which are not only capable of producing generalized tuberculosis by subcutaneous inoculation upon calves and hogs, but will even produce their death in a short time, exactly as many of the bovine cultures would do.

In a recent address before the Medical Association of Leipzig, Eber (1906)⁷ gave the results of a series of experiments which had been carried on under his direction. In this investigation eight cultures of tuberculosis of human origin were tested upon calves ranging in age from two to three months. Of this number two proved to possess only very slight virulence for cattle, two others were of medium virulence, and three were possessed of strong virulence. The eighth culture was found to be without virulence for guinea pigs, and it was therefore deemed useless to test it on cattle. Four cultures derived from cattle were tested upon cattle by methods similar to those used with the human cultures in the same experiment, with the result that two of them were found to be of very slight virulence for cattle, one of medium degree, and only one of a strong degree of virulence. The interesting fact was here demonstrated that it was not an easy matter to cause the infection of cattle with typical tuberculosis by the use of tubercle bacilli derived from cattle, even though the injections were made intravenously.

Goodale (1906)¹⁰ states that within two years he has had under observation seven cases of tuberculous glands of the neck of children from whom he removed the tonsils. On clinical inspection the tonsils showed very little that was abnormal, but on microscopic examination tubercles and giant cells were found. The tonsils were inoculated into guinea pigs, which died subsequently of tuberculosis. Cultures from four of these pigs have been kept under observation by Theobald Smith, who reports that they are cases of bovine tuberculosis. The bacteriology of the cases will be reported in detail by Doctor Smith.

ORIGIN OF CULTURES USED IN PRESENT INVESTIGATION.

The tuberculous material which has been available for the work reported in the present paper has been remarkably varied. There has at no time been a lack of tuberculous tissues from cattle, sheep, or swine, and the supply of similar tissues from human subjects, although not so readily available, has still been ample. The one regrettable feature about the latter material was the inability to obtain as full a history and post-mortem notes on some of the subjects furnishing the cultures for this investigation as could be desired. However, these notes have been made as complete as possible by interviewing members of the families interested, by information gratefully received from the family physicians, or by personal observation.

In addition to the affected specimens from man and from cattle, sheep, and swine, which are commonly made the subject of such comparative studies, many unusual sources have recently been found, and in consequence we have been able to study the characteristics of tubercle bacilli derived from natural infections of the dog, the parrot, the snake, the beaver, the raccoon, the peccary, the agouti, the lemur, the bear, and several varieties of deer and monkeys. The cultures from the wild animals have been recovered more recently and too late to be included in this comparative work; a study of their peculiarly interesting characteristics will form the basis of another paper.

A brief résumé covering the history and such post-mortem notes as were available of the subjects furnishing cultures for this investigation is given below:

1. *Girl I.*—This material consisted of a caseous mesenteric lymph gland of a 6-year-old girl who died of generalized tuberculosis (numerous lesions being found in both the thoracic and abdominal cavities) at the Jefferson Medical College Hospital in Philadelphia. It was impossible to obtain any history of this case except that the child was of sound parentage. The tissue was kindly furnished by Dr. Randle C. Rosenberger, bacteriologist to the hospital, to whom we hereby offer our sincere thanks for this as well as for the material, history, and post-mortem notes of cases designated Girl II, Boy VI, and Case XIV.

2. *Girl II.*—The tissue used in this experiment was obtained from a tubercu-

lar cervical lymph gland in a 13-year-old girl who was successfully operated on at the Jefferson Medical College for scrofula. We are unable to give the subsequent history of the case, as the patient passed from observation.

3. *Sputum A.*—This specimen was recovered from the sputum of Mrs. A., 35 years old, who was suffering from a mild form of pulmonary tuberculosis. The patient removed from the neighborhood immediately after her attack, but is still alive and greatly improved.

4. *Sputum W.*—This sputum was obtained from Mr. W., who removed to Texas in consequence of an attack of phthisis. No report is available as to his present condition.

5. *Boy V.*—The material used for this investigation consisted of the caseous mesenteric lymph gland of a 4-year-old boy who died of generalized tuberculosis. The family history was negative, the other children of the family being entirely healthy. The child developed anorexia, abdominal pains, diarrhea, and abdominal distention, together with a high fever. He had a slight cough, became very much emaciated, and died about six weeks after the development of the symptoms. This child was evidently affected with primary intestinal tuberculosis, as the foci in the lungs were very small and widely scattered and involved only the apices. The spleen and the liver, especially the latter, were likewise sprinkled with tubercular foci. The peritoneum, the omentum, and the mesenteric glands contained many hemorrhagic tubercles, causing numerous adhesions between different portions of the intestines. Four or five ulcers were noticed in the intestinal tract, especially in the vicinity of the transverse colon. Many of the mesenteric lymph glands were swollen and more or less caseated. For this material and the post-mortem notes we extend our grateful thanks to Dr. W. R. Brandenburg, formerly on the staff of Garfield Hospital, Washington, D. C.

6. *Boy VI.*—This culture was recovered from the cervical lymph gland removed at the Jefferson Medical College from a 6-year-old boy. The child was suffering with general lymphatic enlargements (cervical, maxillary, and inguinal) for several months preceding extirpation of the cervical nodes; but subsequent history of this case was not obtainable.

7. *BB.*—Students of the comparative virulence of human and bovine tubercle bacilli are already so well informed in regard to this culture through the writings of Ravenel that a detailed description of its origin is quite unnecessary. Suffice it to say that the culture was isolated by Ravenel from the mesenteric gland of a child whose death was due to tubercular meningitis, which was considered to have been of intestinal origin. Our grateful thanks are hereby extended to Doctor Ravenel for permitting the use of this culture in our comparative work.

8. *Sputum C.*—The bacilli of this culture were isolated from the spleen of a guinea pig inoculated with the sputum of a young man 20 years of age, who was in the last stages of acute pulmonary tuberculosis. He was tall and athletic in build and had a negative family history. His health had been excellent until seven weeks prior to death, when he was attacked by la grippe, which developed into pleuro-pneumonia. During the sixth week of illness tuberculosis was suspected, and a sample of sputum was brought to one of us for examination. Six days later death occurred from phthisis florida. No autopsy was made.

9. *Sputum E.*—The material from which a culture was obtained for this study consisted of sputum from Mr. E., colored, an employee of this Department, who is suffering with chronic pulmonary tuberculosis. He comes from a tuberculous family and has shown symptoms of the disease for the past three years, although at present in a fairly good state of nutrition.

10. *Lung X*.—This specimen was obtained from the lung of a cadaver which was a subject for dissection at a local medical college. Death was due to cerebral apoplexy. The subject appeared to be that of a 55-year-old man, whose previous history was not available. Many foci of tuberculosis were found in both lungs, but were quite scattered and evidently bore no relation to the cause of death. The cheesy pus from a nodule the size of a hazelnut in the apex of the right lung was the material used in obtaining the culture.

11. *Case XI*.—This culture consisted of tubercle bacilli obtained from the pus of an umbilical fistula in Mrs. J. The wound started as a small abscess at the lower border of the navel, which was evacuated and treated aseptically. After three months a similar development occurred which, despite a repetition of the above treatment, persisted for several months during the summer, but it was finally healed. Its recurrence four months later was the cause for a radical operation. The patient was etherized and the abscess, together with 1½ inches of the fistulous tract extending into the abdominal muscles, but not involving the peritoneum, was excised and the wound sutured. No recurrence has thus far occurred, nor is there any indication of tuberculosis elsewhere in the body. The family history is negative, and the patient appears in robust health. In the opinion of the family physician the case must be considered as one of localized infection.

12. *Sputum D*.—This culture was obtained from the sputum of Mr. D., 60 years of age, whose attack with caseous pneumonia about twelve years ago resulted in a small cavity formation in one lung, together with pleuritic adhesions. Several years ago, after an attack of bronchitis, mucopurulent expectorations occurred, associated with more or less hemorrhage. An examination of a small necrosed piece of tissue which was expectorated during a paroxysm of coughing revealed tubercle bacilli, which were obtained on blood serum in pure culture from the spleen of an inoculated guinea pig. The patient three years ago removed to a high, dry altitude for the benefit of his health, and during the past year three microscopic examinations and inoculation tests of his sputa have resulted negatively in each case.

13. *Sputum H*.—This material consisted of sputum from Mr. H., 53 years of age, who was in an advanced stage of chronic pulmonary tuberculosis. The patient had been ill for the past six years, and on examining the sputum myriads of tubercle bacilli were observed. He continued to grow worse until death occurred, seven months after the receipt of his sputum. No autopsy was performed.

14. *Case XIV*.—The culture recovered for this study was obtained from a mesenteric gland of a 2½-year-old man, a pauper, who died at the Philadelphia hospital from generalized tuberculosis. It was impossible to obtain any previous history of this subject. The post-mortem examination showed the lungs to be permeated with numerous areas of tuberculosis, as were the bronchial and mediastinal lymph glands. The liver likewise showed a few isolated foci. The small intestines contained several tubercular ulcers elongated transversely to the axis of the intestines, these lesions being evidently secondary to the pulmonary lesions. Several nodes of the chain of the mesenteric lymph gland were enlarged and more or less caseated. One such gland formed the sources of the emulsion used in the inoculation of a guinea pig, from whose spleen a pure culture on dog serum was obtained.

15. *Case XV, a and b*.—These specimens were isolated from the urine of a 32-year-old man at two periods five weeks apart. The man, who was affected with pulmonary tuberculosis, sought relief from an intense pain in the lumbar region, irregular fever, and slight cachexia. Tuberculosis of one or both kidneys was suspected by his physician, Dr. C. McPherson, to whom we extend thanks

for the material furnished. Culture *a* was derived from the urine voided from the bladder, while culture *b* was obtained from the bloody urine secured by catheterizing the right kidney. The urine from the left kidney failed to show any tubercle bacilli. No further history of this case has been obtained.

16. *Sputum B.*—This sputum was obtained from Mr. B., 21 years old, who was affected for two years with pulmonary tuberculosis. His mother, a brother, and a sister had died of the disease, and another sister was similarly affected. This patient is still alive, and seems to be in better health after his sojourn for two years in the mountainous region of North Carolina.

17. *Sputum G.*—The culture used in this experiment was recovered from the sputum of Miss G., 18 years of age, whose family history is negative. Miss G. is affected with pulmonary tuberculosis, and is gradually growing worse, despite the best of home treatment.

18. *Sputum L.*—This material consisted of sputum from Miss L., 22 years of age, who died of acute phthisis five months after the receipt of this sputum. No autopsy was held.

19. *Monkey.*—This culture was recovered from the spleen of a guinea pig inoculated with an emulsion of the lung of a monkey (rhesus). About November 17, 1901, a large tubercular abscess containing pus appeared in the sub-maxillary region, which was lanced by a veterinarian and the pus scraped out. The monkey was treated by giving cod-liver oil and provided with a large, well-ventilated cage, receiving the best of attention. The principal diet consisted of fruits, and the animal was particularly fond of bananas, hemp seed, and sunflower seed. At no time was it fed on meat, and it received only two bottles of sterilized milk just before death. While sick it drank considerable water and gradually wasted away, and declined in health until death resulted on February 15, 1902. The creature was 2 years old and weighed 10½ pounds. Post-mortem examination showed enlarged caseated right retropharyngeal lymph gland and extensive tuberculosis of the lungs, with involvement of the bronchial and mediastinal lymph glands. The liver also contained a few areas of tuberculosis, and several mesenteric lymph glands were swollen, with cheesy foci present. No ulcers were observed in the intestines.

20. *Bovine S.*—This culture was obtained from the lymph gland of a guinea pig which had been inoculated with a portion of a mesenteric gland of a cow condemned for generalized tuberculosis at a Philadelphia abattoir. On post-mortem examination the lungs were found to be studded with areas of tuberculosis varying in size from a hazelnut to a hen's egg, many of which had coalesced into larger clumps of affected tissue. The entire chain of mediastinal lymph glands was swollen and caseo-calcareous, while the bronchial glands were distinctly gritty, dry, and enlarged. The liver contained a few yellowish foci, while several mesenteric glands were swollen and cheesy. A few grape-like tubercles were found on the omentum and attached to the peritoneal side of the diaphragm. The carcass was poor in nutrition and was that of a cow about 10 years of age.

21. *Bovine T.*—This material was kindly furnished by Dr. J. P. Turner, veterinarian of the District of Columbia health office, to whom we extend our thanks. It consisted of the lung tissue of a young cow 5 years old that was condemned for pulmonary tuberculosis at Benning, D. C. Tubercular lesions were found scattered through both lungs, causing almost a complete solidification of the superior border of the left principal lobe. The bronchial and mediastinal glands were likewise involved, especially the posterior mediastinal, which was as large as a goose egg and caseo-calcareous.

22. *Bovine F.*—This culture was obtained from a tubercular abscess found in

an excavation of the body of one of the cervical vertebra of an aged cow slaughtered at South St. Joseph, Mo., and condemned as unfit for food owing to the generalized and extensive tubercular lesions present. The cervical and thoracic lymph glands were all involved, while both lungs contained innumerable areas of tuberculosis. The serous membranes lining the thoracic and peritoneal cavities showed irregular clusters of pearly bodies scattered unevenly over their surfaces, and a few tubercular areas were also found in the liver and in several mesenteric lymph glands. The previously mentioned abscess of the cervical vertebra was observed on splitting the carcass.

23. *Bovine Y.*—The tissue from which this culture was obtained consisted of a mesenteric lymph gland of a cow about 8 years old affected with extensive and generalized tuberculosis. The retropharyngeal, bronchial, and mediastinal lymph glands were all enlarged and more or less calcareous. The lungs contained many isolated and confluent zones of tuberculosis, at least two of which were in direct communication with the bronchi. The liver showed six foci of tuberculosis, while the surface of the spleen was sprinkled with several nodular masses of tubercular tissue hemorrhagic in character. Two mesenteric lymph glands were slightly enlarged and showed several hemp-seed-like foci of caseous material containing tubercle bacilli.

24. *Hog O.*—This culture was recovered from the spleen of a guinea pig inoculated with a mesenteric lymph gland of a hog which was condemned on post-mortem examination at a Chicago abattoir for generalized tuberculosis. The cervical, bronchial, and many mesenteric lymph glands were tubercular. The lungs contained miliary foci of tuberculosis. The liver and the portal lymph gland showed foci of tuberculosis, while the spleen was enlarged to twice its size and contained numerous isolated areas of tuberculosis throughout its parenchyma. The hog was about 1 year old and was received from the West, but whether it had been fattened on the product of the dairy or otherwise was impossible of determination.

25. *Hog K.*—This material was received from an abattoir in Quincy, Ill., and consisted of a submaxillary lymph gland from a hog otherwise unaffected with tuberculosis. The hog was young, in good condition, and the carcass passed for food. No information as to its origin or previous existence was available.

26. *Hog St. E.*—This tissue consisted of the gastric lymph gland of a hog which had been fed by Dr. E. C. Schroeder, superintendent of the Bureau Experiment Station, on the milk of cow 74, from the St. Elizabeth Asylum herd of cattle used in a previous milk-feeding test by this laboratory. The hog began feeding on the milk November 23, 1900, and continued to receive this food until its death, May 2, 1901. The post-mortem by Doctor Schroeder showed the carcass to be in good condition. The lungs contained many tubercular foci, several of which had become confluent. The mediastinal glands were much enlarged and contained many necrotic points of a tubercular character. The liver was well sprinkled with tubercular foci, while the gastric glands were much enlarged and on section were found to contain a large number of tubercular points of a caseous character. Several tubercular nodules were observed on the parietal pleura.

27. *Sheep M.*—This culture was obtained from the superficial inguinal lymph gland of a guinea pig injected with a portion of a tubercular mesenteric gland of sheep 57 which had been born and raised at the Bureau Experiment Station. This sheep had been used in an experiment with a venereal disease of horses, and showed on post-mortem examination three tubercular mesenteric lymph glands. No other lesions of tuberculosis were detected. This was evidently a case of natural infection through the digestive tract.

THE CULTURES SELECTED FOR THE TESTS.

In the comparative study of the above material no attempt has been made to carry each culture through all the species of experiment animals which were used with some of the cultures. After studying their growth and morphology and inoculation results on guinea pigs and occasionally on rabbits and cats, the cultures were grouped as a matter of convenience for further investigation, and a representative of each group was carried along and used in the inoculation experiments on larger animals. Thus this preliminary work indicated that there was no appreciable difference between eight of the sputum cultures, the two specimens of the urine culture, and the monkey culture, and therefore the latter culture was selected for continuous work, inasmuch as the remaining sputum culture (C) possessed characteristics somewhat unlike the others, and for this reason was retained for more extended study. Of the eight cultures from tubercular human tissues, BB culture was used to compare results with those of Ravenel, Boy V culture was retained owing to its greater virulence, while cultures Girl I and Case XIV were taken from the six remaining cultures owing to their being considered fit representatives of this group.

This method was adopted on account of the desire to obtain as many cultures as possible for comparison, as it was thought needless to carry so many different organisms through a series and subseries of cultures and of various animals, when they could be grouped tentatively and a representative selected for more detailed investigation. That this is not the most desirable method to pursue will be shown by reference to Case XIV culture, which, although at first similar in growth, morphology, and virulence to Girl I culture of this group, later showed characteristics peculiar to itself, especially with reference to its virulence for dogs. It is probable that others might have possessed similar variations from the ordinary if carried through many species of animals, but such work would have been too extensive to have been performed successfully along the lines we have followed, although it opens the subject of the possibility of determining many points of variation more or less persistent between tubercle bacilli of similar origin if injected into many species of animals.

In selecting a bovine culture for comparative work, the pathogenesis of hog cultures O, K, and St. E, and sheep culture M, and the bovine cultures F, Y, and T indicated that they were all of a high virulence and probably all of the bovine type, so that Sheep M and Hog O were taken as representatives, not only because of their similarity in morphology, cultural characteristics, and pathogenesis to the bovine organism, but furthermore on account of their origin in differ-

ent species—sheep and hog. The remaining bovine culture—S—was retained for a more exhaustive study owing to the greater ease and rapidity with which it developed on dog serum and to its milder virulence for rabbits than was shown by the other bovine cultures.

All the above cultures were isolated at various times within twenty-one months, but those carried through the entire experiment were practically of the same age, the isolation of all falling within a period of fourteen weeks. This similarity in age was also a factor, other things being equal, in selecting the representative culture.

Cultures from a dog, a parrot, and several wild animals have been recovered more recently, and although too late to be included in this comparative work, a familiarity with their characteristics has no doubt assisted in extending our knowledge upon which to base general conclusions.

TECHNIQUE OF THE EXPERIMENTS.

ISOLATION OF CULTURES.

The methods employed in obtaining uncontaminated cultures of tubercle bacilli from the original tubercular specimens and the media used for the development of these cultures were as similar to those suggested by Smith in his comparative study of tubercle bacilli as it was possible to adopt.

In order to procure pure cultures from the various tubercular fluids (sputa, pus, and urine), suspensions in physiologic salt solutions were obtained and inoculated subcutaneously into guinea pigs ranging from 330 to 480 grams in weight. In the case of tubercular tissues small sections of the affected material were placed, with aseptic precautions, beneath the skin of guinea pigs. As a result of the protective agencies of the body of the animal, contaminating organisms were usually eliminated before the more virulent and slower-growing tubercle bacilli had become fully established. This mode of separation has given very satisfactory results. The guinea pig is so susceptible to all forms of tuberculosis that it sooner or later gives evidence of the encroachment of the disease, no matter which type is being tested. These animals may be chloroformed to prevent secondary infections as soon as they begin to give evidence of wasting, which was the rule in our experiments, or they may be kept until the disease terminates fatally.

With the more virulent bacilli this loss of flesh was observed about the third week, while the less pathogenic organisms required five or six weeks and sometimes longer. At the necropsy bits of tubercular tissue, usually from the spleen or a distal lymph gland, were removed under the strictest aseptic precautions and transferred to culture tubes containing previously prepared dog serum. The blood

was obtained from the femoral artery of healthy dogs by the observance of the usual aseptic measures. The animal is etherized and the artery is exposed and punctured by a sterile glass rod conveniently bent, through which the blood is conducted into sterile Erlenmeyer flasks. The serum expressed from the resulting clot is drawn up in sterile pipettes, distributed into Salmon tubes, and hardened in a serum oven at 76° C. on three successive days. These tubes are always kept inclined at an angle of about 10°. The resulting medium is not as hard and dry as when a higher temperature is used, nor is it so soft as to be easily broken when sown with tubercular material. After the first two weeks of incubation the tubercular tissue on the serum is crushed against the side of the tube with a heavy flat platinum needle and spread over the surface of the medium for the purpose of starting fresh colonies. Whenever these procedures result in a pure growth of the tubercle bacilli upon the serum the investigator is supplied with the desired culture, and he can then proceed to take up his comparative study of the organism. For the purpose of providing uniform conditions, one incubator only was used, and where possible the transfers, which were made every four to six weeks, were inoculated into tubes containing media from the same batch of serum. The incubator was kept at from 37° to 38° C. and contained a vessel of water to preserve a moist atmosphere.

PREPARATION OF SUSPENSIONS FOR INOCULATIONS.

The difficulty in obtaining a method by which a uniform quantity of tubercle bacilli will be injected into each species of experiment animals is manifest, but the use of a suspension of an opacity similar to that possessed by a 24-hour-old peptone culture of typhoid-fever bacilli has been adopted, as suggested by Smith. When the tubercle bacilli have developed sufficiently on the dog-serum medium certain portions of the surface growth are scraped off by means of a stout, flat, platinum needle and transferred to a dry sterile tube. The masses of growth are minutely broken up against the sides of the tube, which process is aided by an occasional drop of sterile fluid. When well divided sufficient physiologic salt solution is added to make the resulting mixture conform to the cloudiness possessed by the standard typhoid-fever culture. After the heavier particles have settled, the supernatant fluid is used for inoculation. As a further check on the quantity of tubercle bacilli injected, cover-slip preparations were always made to determine more accurately the degree of pollution present. This method was always adhered to with all culture inoculations which were used in the first series of the various species of animals inoculated. An attempt was made to continue the inoculation of culture suspension into the subseries of animals, but the time consumed in awaiting the development of col-

onies, the failure of some tubes to show growth, and occasional contaminations indicated that this course, while highly desirable, was rather impracticable, and several times would have resulted in the loss of the particular organism had not inoculation of animals been made simultaneously with the transfer on culture media. For this reason inoculation of animals after the first series was made by making an emulsion in salt solution of tubercular tissue from the spleen, a distal lymph gland, or other metastatic lesions, in order to insure against any contaminating organism; and freedom from contamination was always determined, so far as a microscopic examination permitted, before the injection.

DOSAGE.

In selecting the doses to be given to the various species of experiment animals an endeavor was made not only to give a uniform quantity of tubercle bacilli to each species, but to give well-proportioned amounts also. Thus cattle received subcutaneously 4 c. c. of either the culture suspension or the tissue emulsion, 2 c. c. being injected in front of each shoulder. This location was selected because of the advantage to be derived from noting whether the bacilli spread from the seat of inoculation to the adjacent pre-scapular lymph glands. The gradual enlargement of these glands is an indication of the virulence of the injected organism. As is well known, bovine tubercular lesions frequently fail to reveal as many bacilli on microscopic examination as similar human lesions show. This condition of sparsity of bacilli occasionally happened when it was desired to obtain the definite amount of tubercle bacilli in an emulsion from a preceding case of tuberculosis, especially a case inoculated with bovine tubercle bacilli. In some cases it was necessary to examine emulsions from several lesions at different locations before a fluid sufficiently polluted with the virus could be obtained, while in other cases, although the usual quantity of suspension had been injected, the total number of bacilli present was lower than the standard. This condition may account for several apparent discrepancies which will be referred to further on. Goats and sheep were similarly injected with 2 c. c. of tubercular fluid, 1 c. c. in front of each shoulder. The failure to obtain enough goats for this experiment necessitated the substitution of sheep for the subseries of inoculations. Dogs were inoculated with a like quantity, in a similar location. Cats received 1 c. c. of the virus, some subcutaneously, but the majority intrathoracically. Rabbits were injected with 0.5 c. c. subcutaneously, on the side of the chest, a few being injected with this size dose intravenously. Mice received 0.1 c. c. of the tubercular virus subcutaneously, at the base of the tail.

STAINING OF BACILLI.

In all of the work of preparing mounted specimens for examination during this investigation the aim has been invariably to stain in a uniform manner all slides that are to be examined comparatively. Carbol-fuchsin has always been chosen as the bacterial stain, and methylene blue has been relied upon for counter staining. In staining tissue for histological study the sections were placed in a large watch glass, covered with cold carbol-fuchsin, and allowed to remain overnight at room temperature under a bell jar. The following morning they were removed from the stain and placed for two minutes in a 20 per cent aqueous solution of sulphuric acid. They were then counter stained with alkaline methylene blue, dehydrated in alcohol, cleared with xylol, and mounted in Canada balsam. In staining bacilli from the various cultures or from lesions of the experiment animals in cover-glass preparations, a small quantity of the surface growth from each of the tubes containing bacilli to be compared, or a similar quantity of tuberculous tissue, is placed upon a separate cover slip and spread out thinly and evenly. After being allowed to dry under a bell jar at room temperature it is fixed by flaming, and carbol-fuchsin is then applied for one-half hour. It is then decolorized by a 20 per cent aqueous solution of sulphuric acid from ten to thirty seconds and counter stained in alkaline methylene blue. Comparisons were always made between bacilli of the same age of development, generally taken from the surface of the same batch of serum, fixed and stained at the same time and by the same methods, thus reducing artificial variations to a minimum.

APPEARANCE OF CULTURES ON DOG SERUM.

Cultures of mammalian tubercle bacilli when grown upon dog serum present at the beginning either a fine, thin, veil-like growth which extends and thickens more or less rapidly toward the formation of a whitish concretion which may assume later the appearance of warty, scale-like, or wrinkled masses, or the original growth appears as delicate, scattered points which gradually extend and increase in size until they may finally coalesce into masses similar to those resulting from growths which were first of the appearance of thin layers. In either case the typical culture of mammalian tubercle bacilli gives no suggestion of a slimy or viscid mass, such as is often produced by avian cultures.

The early growths from tubercular tissues frequently appear very tardily, and their growth for a few generations is feeble and reluctant. As a rule cultures which develop but tardily and scantily will be found possessed of virulence greater than that held by cultures of prompt and luxuriant development, and if submitted to microscopic examination the individuals will commonly be shown as short, straight, evenly staining rods. Hence the culture present-

ing on serum a scanty, tardy growth may be considered as being culturally representative of the bovine type, while the one which exhibits a rapid, luxuriant multiplication may be taken to represent the usual cultural attitude assumed by the human tubercle bacillus sown upon solidified serum.

Our Girl I culture, and the others examined in this test, of which this was selected as a representative, grew rapidly and bulkily when first recovered from the animal tissues. Subcultures from Girl I made perceptible increase in size by the fourth or fifth day, and gradually extended all over the surface of the medium, presenting a white, granular appearance. Cultures recovered after passage through the rabbit were similar to the original cultures in appearance, and after artificial cultivation for three years the most marked changes were a tendency toward a more pronounced grouping or heaping up in the manner of growth, and to a less diffuse vegetation.

The Boy V culture presented quite a contrast to the culture just described, in that it grew very slowly at first and only acquired a capacity for rapid multiplication on serum after three or four generations had been obtained. Even at its best it would not produce such thick masses of substance as were grown by the Girl I culture, but developed upon the surface of the culture serum as a thin whitish layer, thickly sown with small globules or granules. After repeated passage through a series of dogs and of cats the cultures recovered in both instances from these animals showed very plainly an increased tendency toward the production of large, white colonies raised above the surface of the serum, as though the addition of material to the little mounds or heaps was being gradually forced into them from beneath. Cultures recovered after passage through cattle and sheep were very similar in appearance to the original stock culture and corresponded to the bovine cultures upon serum.

Culture BB, isolated by Ravenel, showed practically no difference from culture Hog O in its cultural appearance. The culture received at this laboratory was the twelfth generation, and had already become accustomed to growth upon artificial media to such an extent that subcultures showed an increase of substance by the eighth day after transfer. The passage of BB through the cow, the cat, and the sheep produced no appreciable alteration in its mode of growth, and at all times the cultures recovered from these test animals showed close relationship to cultures which manifested the typical bovine appearance.

Culture Sputum C made only a very reluctant growth when first isolated, requiring three weeks or more for evidence of growth in subcultures. Later subcultures grew more readily, except those recovered after passage through cattle and sheep. These presented very tardy growths for more than a year after being recovered and

isolated, always showing a thin, veil-like form of growth, with a sprinkling of minute white granules scattered over its surface. These bore a very close resemblance to typical bovine cultures.

Case XIV has produced a wide variety of cultures, so far as macroscopic appearance is concerned. Instead of growing in dry, scaly masses, as tubercle bacilli of the human type are accustomed to do, this particular culture has shown more or less of a tendency to spread out over the surface of the culture media as a moist, uniform growth, somewhat after the manner of avian cultures, although it constantly refused to grow with the luxuriance which is usually observed in cultures of the avian type.

The monkey culture was at all times true to the characters of human cultures. The original growth appeared on the sixth day and developed in dry, scaly masses of whitish color and considerable depth. Transfers to subcultures showed prompt and luxuriant development.

Culture Bovine S was at all times a reluctant grower. Although considerably less virulent than Bovine T culture, it proved, contrary to our expectations, to be much more difficult to cultivate. It required almost five weeks for the original growth to develop. When successful cultures were obtained they appeared as fine, disconnected granules of slow development. Subcultures remained apparently inert for three to four weeks, even after many generations.

Hog O and Sheep M cultures were very similar in appearance to each other, and also to Bovine T culture. They all grew promptly in subcultures after the original growths were obtained, and produced fine, granular colonies of small size, scattered over the surface of the solidified serum in great profusion. The typical "ground-glass" appearance so frequently noted in the mention of bovine cultures was plainly in evidence in all of the cultures of these three varieties.

A careful examination of numerous cultures and subcultures of the above organisms, covering many generations during a period of four years, has resulted in the classification of these growths into three classes. A study of these gross appearances was always made upon cultures of the same age, inoculated with a relatively similar quantity of organisms upon the same batch of dog serum, kept in one incubator under precisely the same conditions.

The first class includes those cultures which produce on the surface of dog serum a thin, dry, scanty growth which has been described as peculiar to bovine bacilli, and contains human cultures Boy V, BB, and Sputum C, and bovine cultures S and T, Hog O, and Sheep M. It will be observed, however, that Sputum C culture does not conform in morphology and virulence to the bovine type, although it was found to have far greater pathogenicity than is usual for sputum bacilli.

DESCRIPTION OF PLATE I.

- FIG. 1. Human tubercle bacilli, Boy V. Dog-serum culture, second generation, 19 days' growth.
- FIG. 2. Human tubercle bacilli, BB. Dog-serum culture, twelfth generation, 19 days' growth.
- FIG. 3. Human tubercle bacilli, Boy V. Dog-serum culture, sixteenth generation, 19 days' growth.
- FIG. 4. Human tubercle bacilli, Boy V. Dog-serum culture, first generation, 19 days' growth, after passage through sheep No. 104.
- FIG. 5. Human tubercle bacilli, Boy V. Dog-serum culture, fifth generation, 19 days' growth, after passage through 15 cats.
- FIG. 6. Human tubercle bacilli, Boy V. Dog-serum culture, second generation, 19 days' growth, after passage through 5 rabbits.

DESCRIPTION OF PLATE II.

- FIG. 1. Human tubercle bacilli, Case XIV. Dog-serum culture, second generation, 18 days' growth.
- FIG. 2. Human tubercle bacilli, Case XIV. Dog-serum culture, second generation, 18 days' growth, after passage through sheep.
- FIG. 3. Human tubercle bacilli, Case XIV. Dog-serum culture, first generation, 18 days' growth, after passage through 3 dogs.
- FIG. 4. Bovine tubercle bacilli, Sheep M. Dog-serum culture, original culture, 23 days' growth.
- FIG. 5. Bovine tubercle bacilli, Sheep M. Dog-serum culture, second generation, 23 days' growth, recovered from the seventh of a series of rabbits.
- FIG. 6. Bovine tubercle bacilli, Sheep M. Dog-serum culture, fourth generation, 23 days' growth, after passage through a series of 6 cats.

DESCRIPTION OF PLATE III.

- FIG. 1. Bovine S tubercle bacilli. Dog-serum culture, first generation, 19 days' growth.
- FIG. 2. Bovine S tubercle bacilli. Dog-serum culture, thirteenth generation, 19 days' growth.
- FIG. 3. Bovine S tubercle bacilli. Dog-serum culture, third generation, 19 days' growth, recovered from sheep No. 117.
- FIG. 4. Bovine S tubercle bacilli. Dog-serum culture, first generation, 19 days' growth, after passage through 3 rabbits.
- FIG. 5. Bovine tubercle bacilli, Hog O. Dog-serum culture, eleventh generation, 19 days' growth, after passage through 5 cats.
- FIG. 6. Bovine tubercle bacilli, Hog O. Dog-serum culture, first generation, 19 days' growth, recovered from the seventh rabbit in a series.

These cover-glass preparations were stained with carbol-fuchsin and decolorized with 20 per cent sulphuric acid. Drawn with camera lucida at base of stand; Zeiss 2 mm. oil immersion objective and No. 4 compensating ocular, $\times 900$.

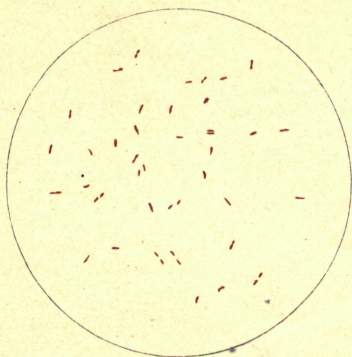


FIG. 1.



FIG. 2.

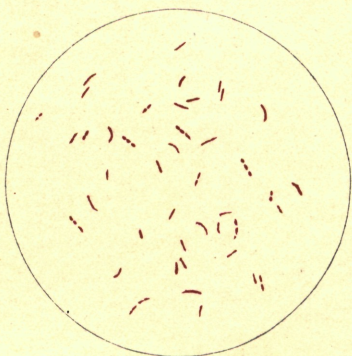


FIG. 3.



FIG. 4.

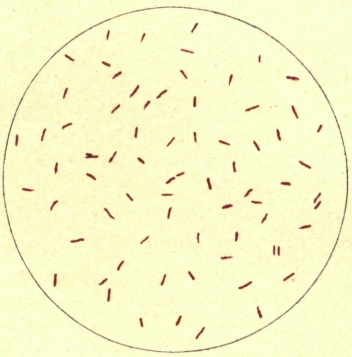


FIG. 5.

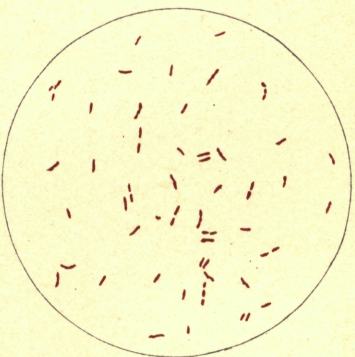


FIG. 6.

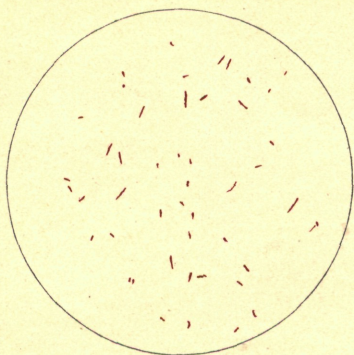


FIG. 1.



FIG. 2.

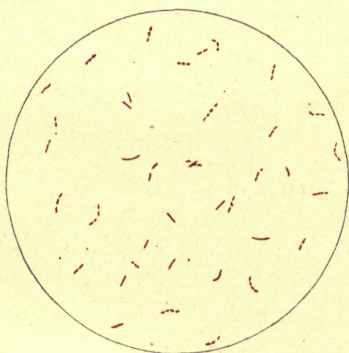


FIG. 3.

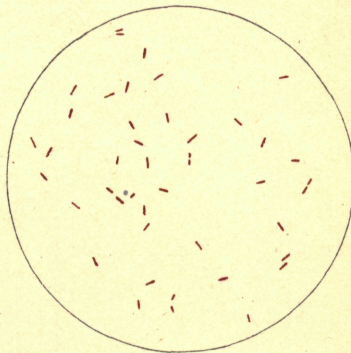


FIG. 4.

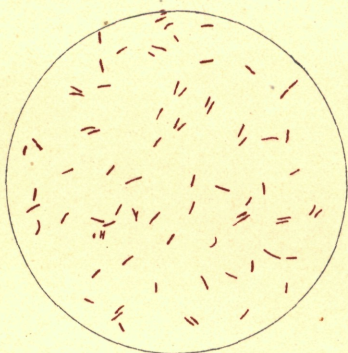


FIG. 5.



FIG. 6.

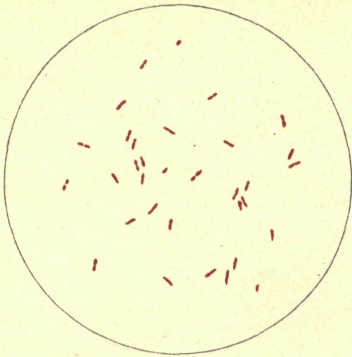


FIG. 1.

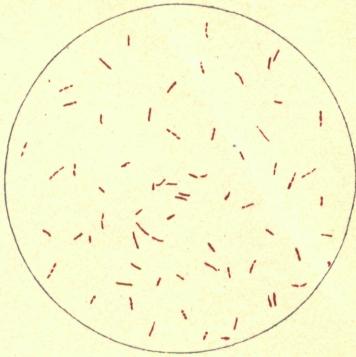


FIG. 2.



FIG. 3.

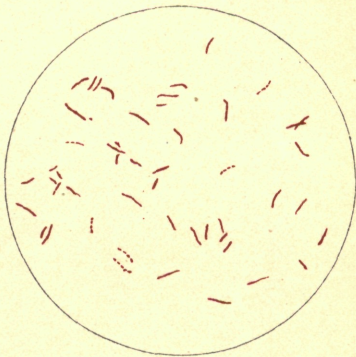


FIG. 4.

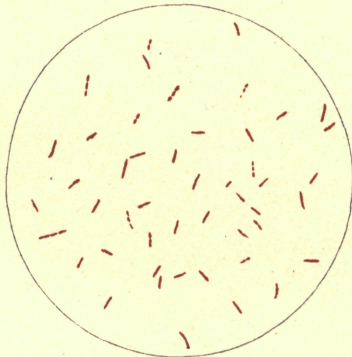


FIG. 5.

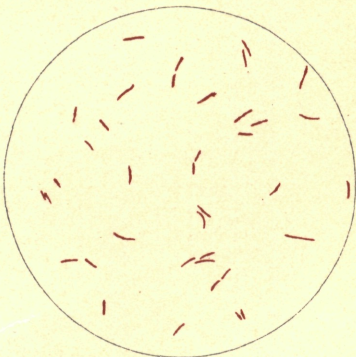


FIG. 6.

The second class presented a profuse growth of quickly appearing colonies, which soon piled up over the surface in luxuriant development similar to cultures described as human types. These cultures include those represented by culture Girl I and the monkey culture.

A third class consisted of one member, Case XIV, which, as described above, showed a moist growth somewhat similar to that accredited to avian bacilli. This culture, however, did not coincide with the avian type in any other respect.

CHARACTERISTICS IN SUBCULTURES.

Judging from the naked-eye appearance of subcultures of the different varieties of tubercle bacilli studied, it may be said that the most noticeable change as one passes away from the original culture is an increased adaptability to growth and multiplication under artificial conditions. The perceptible increase of bulk occurs earlier in the later subcultures than it did in the original, and the bulk finally attained in each tube will, with thrifty cultures, show gradual accession.

MORPHOLOGICAL EXAMINATIONS.

The form assumed by tubercle bacilli when grown upon solidified dog serum is at the present day one of the leading factors in their classification as representatives of a particular type. The statement that a certain culture of tubercle bacilli is of the bovine type does not necessarily mean that it was obtained from some animal of the bovine race, but gives expression to the fact that when examined microscopically the bacilli forming such culture are presented to view as short, straight, uniformly stained rods. Reference to Plate I, figures 1 and 2, will show the morphological character of tubercle bacilli of the bovine type, even though the cultures here represented are of known human origin. On the other hand, tubercle bacilli that are strictly of the morphology of the accepted human type may be met that are traceable to bovine origin.

Girl I culture and the others of its class produced individuals that were 3 μ in length, beaded and slightly curved, with lesser members down to not over 1 μ in length, and that stained evenly. Different slides show a variation in the proportion of long or short forms, but the above extremes show no material deviation. Continuous cultivation of this organism by subcultures on dog serum has failed to affect materially the morphology of this bacillus. Repeated passage of this culture through guinea pigs and rabbits has failed to produce any noticeable alteration in the form of its members, unless it may have been to add slightly to their thickness.

Culture Boy V was a good exponent of the bovine type of tubercle bacilli from the start. Measuring from $1\ \mu$ to $1.5\ \mu$ in length, its individuals were found to be plump, straight rods with even staining. (Pl. I, fig. 1.) Continuous cultivation upon dog serum has apparently given to the individuals of this culture a tendency toward the attainment of slightly greater length. (Pl. I, fig. 3.) In old cultures after the seventh generation they show a greater readiness to produce longer forms than is possessed by the culture Bovine T grown under similar conditions, but no greater than culture Sheep M has shown. Passage of tubercle bacilli from culture Boy V through cattle does not seem to have any effect upon their form. Passage through sheep gives them a slight permanent elongation, without beading. (Pl. I, fig. 4.) On their recovery from the organs of cats or rabbits the individual bacilli are seen to be transformed completely; they are much longer and much slenderer, uniformly beaded, and frequently curved. Returning them to the surface of dog serum they lose much of their added length, but still possess a greater length and more beading than the members of the original stock culture. (Pl. I, figs. 5 and 6.)

Continuous cultivation of Culture BB for three years upon dog serum has not greatly changed the individual micro-organisms, except to make them a trifle more slender. This culture, like the one described above, became greatly elongated by passage through the cat and the rabbit when obtained direct from the tissues, but later cultivation served to shorten them again almost to the starting point.

The statement has been made in the introductory part of this work that culture Sputum C possessed characteristics somewhat different from those of the other sputum cultures tested. When first recovered in pure culture the members were noticeably short for sputum bacilli— 1 to $2\ \mu$ —and there was also an unusual proportion that stained evenly, showing no beading. This characteristic has been lost somewhat during its long cultivation. Beaded forms have come to predominate, increased length is shown, and a greater proportion of the members show curves.

Passage through cattle, sheep, and dogs seemed to fix the short, straight form more permanently upon the bacilli, and subcultures from these cultures showed little inclination to elongate or to become beaded until several renewals had been reached. After passage through cats and rabbits an increase of length and of beading could be determined, which did not entirely disappear upon further cultivation.

The individual bacilli from cultures of Case XIV can not be said to have adhered to any particular type, owing to the intermixture of numerous forms, broader in the center than at the ends, with

others which may be said to represent both the bovine and the human types. The major part of the bacilli shown upon the slides are slender rods, showing a slight tendency toward beading and curving. A few long individuals are also shown, and some short members may be seen in most of the slides examined. (Pl. II, fig. 1.)

Passage through the cow or the sheep does not entirely eliminate the short, irregular forms, but it does remove most of the long, beaded bacilli. (Pl. II, fig. 2.) Recovered from dogs, they appear as very delicate, slender, beaded threads, intermingled with short, evenly stained organisms. (Pl. II, fig. 3.) Further cultivation upon dog serum does not fully remove the variety of forms which are to be found in early cultures. In the body of the cat the bacilli acquire a more uniform length. This culture is somewhat at variance with the others studied, in that it possesses no form or length that may be considered typical.

The monkey culture gave the appearance at all times of a typical sputum culture. The individual bacilli were long, beaded, and often curved. It retained its characters in spite of passage through various experiment animals and of continuous cultivation upon dog serum through many generations.

Culture Bovine S, when first grown in pure culture, appeared as typically bovine so far as morphology is concerned. (Pl. III, fig. 1.) It displayed deviations in virulence and in cultural qualities, however, and later investigations have shown that it will develop members of unusual length, accompanied with more or less beading, rather more readily than was the case with culture Bovine T. Examination of the thirteenth generation of the stock culture of Bovine S shows a material lengthening of the members, together with occasional beading. (Pl. III, fig. 2.)

Passage through sheep also served to elongate the bacilli, as will be seen by reference to a preparation made from the nineteen days' growth of the third generation on dog serum. (Pl. III, fig. 3.) A material extension in the length with frequent beading also follows passage through a series of cats or rabbits, as is indicated by the same aged growth of the same numbered series. (Pl. III, fig. 4.)

Culture Hog O, derived from a mesenteric lymph node of a hog, adhered closely to the bovine type when first recovered, and continued cultivation for three years upon dog serum has caused no apparent changes. As with some of the other cultures, passage of culture Hog O through a series of cats served to give a permanent extension of length (Pl. III, fig. 5), and the effect of passage through the rabbit proved to be very similar. (Pl. III, fig. 6.)

There was never any great difference observable between culture Hog O and culture Sheep M, the latter originating from the mesenteric lymph gland of a sheep. The effects of long-continued cultiva-

tion and of animal passage were very similar upon these two cultures. Plate II, figure 4, shows the original sheep culture after it had begun to make noticeable growth, and figure 5 represents this strain of bacilli recovered from the seventh of a series of rabbits after growth for two generations upon dog serum, while figure 6 of the same plate shows the effects of passage through a series of six cats followed by cultivation for four generations upon dog serum.

REVIEW OF MORPHOLOGICAL RESULTS.

It is interesting to review the results obtained by this study of the morphology of the above cultures on dog serum. We find that a marked difference is shown between bacilli of human origin and also between those organisms obtained from sheep, hog, and bovine. Too great dependence, therefore, should not be placed upon the morphology of organisms alone in determining their origin, although it is now quite generally conceded that marked differences are frequently shown to exist between tubercle bacilli derived from different sources. In recognition of this repeatedly demonstrated fact it has become customary to refer to short, straight, stubby bacilli, which show no beading when stained by the usual methods, as being representatives of the bovine type. Those that appear in various lengths, irregularly curved and beaded, and when grown upon solid media develop in dry, profuse colonies more or less granular, are referred to as being of the human type, while those of somewhat similar conformation to the latter, although longer, that produce a smooth, moist, amorphous growth upon solid media, are considered avian in type. Many cultures will be met, however, which, if judged by the above standards alone, will prove very puzzling to the investigator, and consideration of the species of the host which produced them, together with their cultural characteristics and their virulence toward experiment animals, will not give a positive solution to the problem. Some writers base their classification of tubercle bacilli upon morphology alone and are at once confronted by bovine and human types in a single human cadaver or by a similar combination and various mixtures with the avian type in one of the domestic animals. They explain such occurrences as these by arguing that they are dealing with a mixed infection, and that the patient actually harbors two or more species of tubercle bacilli at one time.

In the course of the investigations here recorded the writers have examined several thousand microscopic preparations from tubercle cultures and feel impelled to state in the light of these numerous presentations that a commingling of different types of tubercle bacilli in a single subject is by no means of rare occurrence if one is to judge by morphology alone. Furthermore, we have noted in

this study that cultures Boy V and BB correspond in every particular to the types spoken of as bovine, yet both were derived from children; while probably the longest bacilli we have recovered from tissues came from the monkey culture and a culture (undescribed in this paper) derived from the tubercular vertebra of a cow. If we adhere strictly to the classification of these bacilli by their conformation, it is necessary to admit that cultures Boy V and BB are bovine organisms infecting human beings, and that the monkey culture and the culture from the vertebral abscess of a cow were human organisms infecting these animals, but that their sojourn in their respective new hosts was not of sufficient length for them to develop those qualities induced by their environment.

It seems perfectly plausible to accept the theory that the effect of the tissues of the human body on any tubercle organism is gradually to elongate it and render it thin and beaded, while the same organism in cattle, owing to its peculiar environment, is shortened and made more solid and stubby. An analogous condition has been proved by various writers in their experiments with tubercle bacilli on culture media. Surely if it be accepted that the tubercle bacillus on egg media is made short, stubby, and solid, and the same organism in glycerin-bouillon becomes elongated and beaded, it can be argued by analogy that the body of cattle is a culture medium which shortens the germ, while the living culture medium furnished by the body of man serves to elongate the germ, as does glycerin-bouillon. Therefore, while we agree that the differences which exist between many human tubercle bacilli and the majority of bovine bacilli are sufficient, when these extremes are met, to classify them tentatively as types or varieties, we must adhere to the belief that both germs originate phylogenetically from a parent stock, and that the differences assumed in the bodies of man and cattle are the result of environment that can be approached by inoculating either germ into various appropriate culture media. Natural infections of cattle, hogs, sheep, horses, cats, fowls, and man have been reported by various authors in which the bacillus has been found to be identical in every respect with those classed as bovine. Here it will be seen that the natural distribution of bovine tubercle bacilli covers a very wide range, including, as it does, ruminants, omnivora, solipeds, carnivora, and fowls. Bacilli of the type recognized as human have been found attacking a still wider range of subjects, while bacilli of the avian type have been isolated from horses, cattle, rats, mice, man, and very many species of birds.

The more this subject is studied the more numerous are the instances noted in which the bacilli appear spontaneously in animals that are far removed from the species supposed to be their natural host, thus proving that the fields for their natural infection are not

bounded by any hard and fast lines and that there is no character possessed by any tubercle bacilli in one host that may not be found in tubercle bacilli in another host, under favorable conditions. Is it not therefore reasonable to consider these various types of tubercle bacilli obtained from these different hosts as belonging to the same species of organism and showing in alteration of form the effect of sojourn in the bodies of different animals? May it not also be assumed in those cases where various types of tubercle bacilli are obtained from one animal that these are transitional forms which have not had time or opportunity during their sojourn to become transformed into the usual type peculiar to this host? It is not an uncommon experience in laboratory work to find that certain characteristics are lost by repeated cultivations, and by the continuation of such conditions certain characteristics become prominent which did not obtain in the original culture. Such organisms may be returned to their original characteristics by injecting them into animals, and it is therefore generally stated that these changes are due to unfavorable environment.

A more complete discussion on this subject will be given in another paper on the transformation of tubercle bacilli.

PATHOGENESIS.

VIRULENCE FOR RABBITS.

In the inoculation of rabbits for purposes of comparative study the subcutaneous method was adopted in all cases except with a series of five rabbits used in the work with culture Girl I. The inoculations were made on the left side of the animal, at a point 20 mm. posterior to the supero-posterior angle of the scapula. The amount of material injected was 0.5 c. c. in practically every instance, and careful endeavor was made to have this quantity represent approximately a similar number of bacilli in all cases. All cultures had been recently isolated and were injected in most cases when between 20 and 34 days old, usually of the first or second generation, but occasionally of the third generation. The animals were not disturbed after their inoculation, no attempt being made to take their temperatures.

The results have been collected in tabular form for purposes of ready comparison. (See p. 44.) It will be seen that the first rabbit of each series was inoculated with the pure culture and that each succeeding rabbit was injected with an emulsion in physiologic saline solution of some tubercular tissue from the preceding animal. In preparing the material for the original inoculations great care was used to comply with the conditions outlined by Theobald Smith in regard to the number of bacilli to be introduced at one inoculation.

For this purpose the standard of a 24-hour-old bouillon culture of typhoid bacilli was used in the determination of the density of the suspension of tubercle bacilli injected.^a

The initial weights of the rabbits used ranged from 1,320 to 1,660 grams in most instances. In very exceptional cases it was impossible to obtain rabbits of this size and heavier ones were inoculated.

RESULTS OF RABBIT INOCULATIONS.

In making the initial inoculation with culture Girl I, the material used was the second subculture on dog serum and was of twenty days' growth. The culture was at this time showing a vigorous growth, and the individual bacilli were therefore of necessity in a thriving condition.

From the fact that this first rabbit of the series lived for one hundred and seventy-seven days one must conclude that the Girl I culture is far removed from the bovine type. The lesions developed, however, were scattered throughout the visceral organs and lymphatic glands of both the thoracic and abdominal cavities. Post-mortem examination disclosed at the point of inoculation a subcutaneous abscess about 2 cm. in diameter in the center of which was a small amount of creamy pus.

Rabbit No. 574, second in the series, was inoculated with an emulsion of one of the tuberculous lymph glands of the preceding animal. The advance of the disease was more rapid in this case, and, in addition to the tuberculous ulcer left at the point of inoculation by the rupture of the walls of the inoculation abscess, there was a surrounding zone, some 16 mm. broad, in which the subcutaneous tissues were permeated with small tubercular foci more or less confluent. In this case also the visceral organs of both of the body cavities were very generally involved.

The next rabbit of the series evidently died from the interference of respiration which necessarily followed the serious occlusion of the air spaces of the lungs by the enlargement of the tubercular growths within this organ, as the lesions in other parts of the body were not as far advanced as in the preceding animal, and were not apparently sufficient to have been the cause of death. At the point of inoculation a firm tumor developed, but, instead of degenerating and forming a circumscribed abscess, the growth was dense and fibrous. The lesions of the axillary lymph glands were dry and firm,

^a We were very fortunate in the fact that we were able to leave this work to Dr. J. R. Stewart, who had previously been associated for many years with Smith in his research work and who had thereby become thoroughly versed in his methods. We wish to express our obligations to Doctor Stewart for his valuable assistance rendered in this and many other portions of the work.

with no remaining evidences of inflammatory extension. Kidneys and spleen were just beginning to evidence recent attack.

The inoculation of rabbit No. 824 resulted in a disease of very slow development. At the post-mortem examination, however, it was found that although the animal had increased in weight during the development of the disease, there were but few of the visceral organs or glands that had failed to suffer from attacks of the invading micro-organisms. At the seat of the injection was a firm, dry tumor which showed no tendency toward softening. The costal pleura had a covering of inflammatory tubercular neoformations over a wide area in the regions near the sternum. Lungs, liver, spleen, and kidneys were seriously involved. The average length of life of the above animals after injection was one hundred and eighteen and three-quarters days.

The later rabbits of this series were inoculated intravenously, and further description of them may therefore be dispensed with, as they can not be used in comparison with the rabbits of the other series.

Turning to culture Boy V, we find that in spite of the greatest possible care to make the inoculation similar throughout there was a considerable variation in the results obtained. Rabbit No. 646, receiving the pure culture from dog serum, second subculture of twenty-one days' growth, developed a widespread infection of tuberculosis, which on post-mortem examination was seen to consist of an infinite number of minute foci thickly sown about in the substance of the lungs, liver, spleen, and kidneys, while the various lymph glands of the body were similarly infected, and the intestinal walls in the ileocecal region were thickly studded with small whitish nodules.

Although the period of survival varied from twenty-four to eighty-four days with the rabbits of this series, the picture presented by the post-mortem examination of each one (except the fourth—No. 832—which seemed slightly milder than the others) was that of a virulent, rapidly advancing type of tuberculosis, which readily assumed inflammatory properties, as was evidenced by the presence of a zone of inflammation, more or less broad, surrounding many of the tubercular foci in the various organs. The average period of life of the rabbits of this series was fifty-nine days, which indicates a greater degree of virulence than was possessed by culture Bovine S, in which the average duration of life with the inoculated rabbits reached sixty-seven and five-sixths days.

The material used in testing culture BB upon rabbits was a culture from a tubercular nodule removed from the mesentery of cat No. 43, which was the fifth of its series, as will be seen by referring to the tabular report upon cats on page 51. This variation from the cus-

tomary method of procedure was deemed justifiable because of the fact that BB had already been very thoroughly studied by Ravenel in regard to its action upon rabbits. Rabbit No. 804, which served as the first of the series, lived for sixty-two days following the inoculation, and at autopsy presented lesions very similar to those developed in rabbits by the use of culture Boy V, except that there was a more general extension of tubercular processes to the pleural and peritoneal membranes. This tendency was shown throughout the entire series. The average duration of life with the rabbits of this series was sixty and one-half days, and at autopsy each animal disclosed tuberculosis of a severe and generalized character.

The effect of inoculating culture Sputum C upon rabbits was more surprising. If we discard No. 634 from the calculation because of an accident which necessitated its destruction on the twenty-first day, and No. 741, which was chloroformed on the thirty-sixth day in order to furnish material on that particular date for a comparative test, we find that the average duration of life with this series was fifty-three and one-fifth days. This attests the remarkable pathogenicity of this sample of sputum. The first two rabbits of the series were inoculated with the third subculture from dog serum after growing for twenty-three days upon this medium. At this time the bacilli were noticeably short for sputum bacilli, and there was also an unusual proportion that stained evenly, showing no beading. At the autopsy of No. 521, on the seventy-fourth day, it was shown that the inoculation had produced an open ulcer at the seat of the inoculation. The axillary lymph glands of both right and left sides were badly tuberculous. The thyroid, sublingual, prepectoral, mediastinal, mesenteric, and knee-fold glands all contained tubercular foci, some showing many while others presented only two or three foci. The lungs were very much enlarged, and all lobes showed miliary tuberculosis, being partially made up of a confluent mass of tuberculous foci. The kidneys both contained many tuberculous foci, some of which were quite large and extended above the surface of the renal tissue. The bladder contained a quantity of urine in which considerable flocculent precipitate was present.

Rabbit No. 559, second of the series, was inoculated with an emulsion of a tuberculous axillary gland from the preceding animal and succumbed in thirty-three days. The lesions shown by this animal were very similar in extent and character to those seen in its predecessor. The rapidly fatal termination can not be traceable to the fact that the material used in the inoculation was taken directly from animal tissues instead of from a culture tube, for we find rabbit No. 794, the eighth of the series, surviving for seventy-six days under identical conditions. It appears to be an instance where individual susceptibility played a prominent part. Examining the course of

the disease throughout the entire series, we find that it was in each instance acute and rapid. The bacilli quickly found their way from the point of inoculation to the internal organs and began immediately to exert their destructive influence wherever a foothold was gained. Several of the animals developed tubercular growths upon the pleural surfaces. These were usually flat with broad bases, showing no tendency toward stem formation. An extension of the growth over a longer period of time might have altered the results in this respect.

In the work with culture Case XIV, human, it was found that pure cultures after artificial cultivation were almost without effect upon rabbits. The first rabbit used was chloroformed forty-eight days after inoculation, but showed not the slightest evidence of tubercular infection. A similar result followed the inoculation of a second rabbit, as was shown by an autopsy held fifty-seven days afterwards. The third attempt resulted in the formation of a small degenerating tumor at the seat of inoculation, slight hemorrhagic swelling of the axillary glands, and the formation of numerous small foci in the lungs. An attempt to perpetuate the organisms collected from these lesions by injecting them into a fourth rabbit proved to be a complete failure, as no tubercular alterations resulted.

All inoculations of rabbits with this culture were now suspended for some time, and it was only when an accentuation of virulence had been obtained by passage through a series of dogs that this part of the experiment was resumed. When an autopsy was held upon dog No. 82, the third canine in the Case XIV series, a generalized case of tuberculosis was found, and two rabbits were immediately inoculated each with 1 c. c. subcutem of an emulsion of a bronchial lymph gland prepared in physiologic salt solution. Both of these rabbits soon gave evidence of serious infection, and both developed generalized cases of tuberculosis. No. 884 served as the first of the new series of rabbits. Although living but forty-six days after the inoculation, it gained 56 grams in weight during that time. Nevertheless the lesions were found to be widely scattered when the autopsy was held. At the seat of inoculation a shallow wide-spreading tumor, formed of a collection of various sized caseous nodules, had formed. The axillary glands were enlarged, hemorrhagic, and contained many caseous foci. The thymus glands showed similar changes. Bronchial and mediastinal glands were likewise altered. The lungs were crowded with large, round tuberculous growths, of which those near the surface projected prominently. The kidneys were thickly studded with miliary foci, while the spleen was enlarged, inflamed, and mottled. Lymph glands of the mesentery showed enlargement, and numerous caseous foci were present.

With the degree of pathogenicity now acquired by Case XIV for

rabbits, no trouble was experienced in extending the test through the entire series, with an average duration of life of forty-eight and two-thirds days after the inoculation. The disease assumed a virulent, progressive character in each instance, thus illustrating the possible transformation which may be brought about in tubercle bacilli by passage through animals of a species different from their original host.

In the monkey culture (also the eight sputum cultures, and the two from urine which were classed with it) a type of tubercle bacilli was encountered which possessed only the slightest degree of virulence for rabbits. By the time that this culture had been passed through three rabbits with negative results it had proved to be without pathogenicity for larger test animals of other species, and consequently all further experiments upon rabbits were dropped. Moreover, the attempt which was made to increase its virulence, as was done with culture Case XIV, also resulted negatively.

Every inoculation of rabbits with tubercle bacilli of culture Bovine S resulted in tuberculosis of a generalized character. While its virulence for rabbits was more nearly equal to that of the other bovine as well as the porcine and ovine cultures used than was found to be the case with other species of test animals, there was still a noticeable tendency to form lesions that were lacking in inflammatory character. The slightly greater duration of life after the inoculations is not marked enough to be worthy of comment if conclusions were to be drawn from the rabbit tests alone, but may be taken into consideration in the final estimate of the pathogenicity of this culture. The material used in inoculating the first rabbit of the series was the second subculture of twenty-four days' growth. At the autopsy, seventy-seven days later, the lungs and spleen were found to be apparently unaffected. The axillary glands and the lymph glands of the thoracic and abdominal cavities contained no visible tubercular change. At the seat of the inoculation a circumscribed tuberculous tumor had formed which was of a soft, caseous nature within the surrounding capsule. The costal pleura showed several areas of fibrous inflammation. One-third of the omentum was closely covered with fibrinous neoformations, the more mature of which appeared like small "pearly" bodies. Kidneys and liver contained many miliary foci. In the case of the second rabbit of the series the lungs quickly became involved, and the death of the animal was thereby hastened. The remaining members of the series returned closely to the conditions of the first rabbit, both as to duration of life and as to the character of the lesions produced.

Culture Hog O proved to be very virulent for rabbits. Excluding the second of the series, which met with an accident, the duration of life with the series averaged sixty and five-sixths days. In the post-

mortem examination of each member of the series a rapidly advancing and widely diffused type of disease was shown.

Culture Sheep M produced lesions very similar to those following the use of culture Hog O. The material used at the commencement of the series was the second subculture of twenty-three days' growth. The lesions developed consisted of a degenerating tumor at the seat of inoculation, with numerous minute foci in lungs, spleen, kidneys, liver, and lymphatic glands, which showed how thoroughly the work of carrying the bacilli to the distant tissues had been performed. The average duration of life after injection of these animals was sixty-six and three-sevenths days.

SUMMARY OF RABBIT INOCULATIONS.

If now we endeavor to inspect critically the above results it must appear evident that the individual resistance manifested by these rabbits varied to a great extent. However, the number of animals in each series serves to reduce this influence upon the results to a minimum. In comparing the degrees of pathogenesis of the various cultures used, one of the most striking features is to find the unusual virulence of Sputum C for rabbits, a virulence which was not established with any of the other experiment animals tested. Cultures Boy V and BB are also seen to possess pathogenic powers equal if not superior to the cultures of bovine type. Thus if comparison be made between the first series of inoculations with pure cultures of the various bacilli, or if the entire series in each case be averaged, the conclusion is evident that these three human cultures possess for rabbits by subcutaneous inoculation a virulence greater than is possessed by culture Bovine S, and similar to that shown by cultures Hog O and Sheep M. On the other hand culture Girl I possessed only slight virulence for rabbits, while the original culture of Case XIV and the monkey culture were practically without effect on these animals.

Table showing results of inoculations of rabbits.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of rabbit.	Material used for inoculation.	Days alive.	Change in weight (+gain; -loss).	Result.	Remarks.
				<i>Grams.</i>		
Girl I.....	369	Culture.....	177	-377	G.	Disease widely generalized.
	574	Lesion from 369.....	67	-368	G.	Open ulcer at seat of inoculation.
	748	Lesion from 574.....	58	-679	G.	Very complete involvement of the lungs.
	824	Lesion from 748.....	173	+255	G.	Case of slow progression but of widespread infection.
	1040	Lesion from 824.....	18	-509	G.	Intravenous inoculation.
	1043	Lesion from 1040.....	18	-453	G.	Do.
	973	Lesion from 1043.....	35	-594	G.	Do.
	1100	Lesion from 973.....	20	-396	G.	Do.
	1055	Lesion from 1100.....	21	-113	G.	Do.
Boy V.....	646	Culture.....	64	-453	G.	Lesions consist of numerous small tubercular foci, widely disseminated.
	707	Lesion from 646.....	46	-30	G.	Every indication of rapid inflammatory invasion.

Table showing results of inoculations of rabbits—Continued.

Source of bacilli.	No. of rabbit.	Material used for inoculation.	Days alive.	Change in weight (+ gain; - loss).	Re-sult.	Remarks.
				<i>Grams.</i>		
Boy V	759	Lesion from 707.....	55	+ 226	G.	Lungs and pleuræ most seriously affected.
	832	Lesion from 759.....	81	- 226	G.	Many tubercular fibrous growths attached to parietal pleuræ.
	901	Lesion from 832	24	- 340	G.	Inflammatory form of disease.
	934	Lesion from 901.....	84	- 566	G.	Disease widely generalized.
B B.....	804	Culture from cat 43.	62	+ 56	G.	Severe type of disease.
	847	Lesion from 804.....	79	- 170	G.	Whitish pearly growths upon costal pleuræ.
	935	Lesion from 847.....	46	+ 226	G.	Pearly growths upon costal and mediastinal pleuræ.
	926	Lesion from 935.....	61	- 308	G.	Pearly growths upon costal and diaphragmatic pleuræ.
	1025	Lesion from 926.....	61	- 566	G.	Severe type of disease.
Sputum C ..	1077	Lesion from 1025.....	54	- 538	G.	Generalized and extensive lesions.
	420	Culture.....	59	- 62	G.	Advancing inflammatory type.
	521	Culture.....	74	- 680	G.	Severe progressive lesions.
	559	Lesion from 521.....	33	- 84	G.	Severe type.
	575	Lesion from 559.....	50	- 280	G.	Rapidly advancing disease.
	634	Lesion from 575.....	21		L.	Brog broken.
	650	Lesion from 634.....	58	- 340	G.	Extensive diffused tuberculosis. Lungs firmly adherent to costal walls.
	723	Lesion from 650	35	- 56	G.	Moderate type.
	741	Lesion from 723.....	36	- 112	L.	Chloroformed to gain material for comparative test.
	794	Lesion from 741.....	76	- 30	G.	Widely generalized case.
	858	Lesion from 794.....	42	- 210	G.	Costal and diaphragmatic pleuræ show numerous white tubercular growths.
	680	Lesion from 858.....	55	- 906	G.	Pericardium and costal and mediastinal pleuræ sown with small white tubercular growths.
Case XIV...	962	Lesion from 680.....	50	- 566	G.	Lesions widely generalized.
	853	Culture.....	48	+ 30	N.	Chloroformed.
	844	Culture.....	57	+ 56	N.	Do.
	654	Culture.....	105	+ 170	L.	Chloroformed; very mild form of disease.
	762	Lesion from 654.....	79	+ 283	N.	Chloroformed.
	834	Culture from dog 82.	28	- 226	G.	Chloroformed; progressive spreading case.
	884	Culture from dog 82.	46	+ 56	G.	Progressive spreading case.
	964	Lesion from 884.....	59	- 255	G.	Inflammatory type.
	783	Lesion from 964.....	59	- 509	G.	Widely generalized case.
	894	Lesion from 783.....	57	- 877	G.	Strong growth of pearly bodies upon costal and diaphragmatic pleuræ.
Monkey	1134	Lesion from 894.....	37	- 113	G.	Most serious lesions are in lungs.
	1084	Lesion from 1134.....	34	- 150	G.	Lesions widely generalized.
	373	Culture.....	40	0	L.	Only lesion is at point of inoculation.
	418	Lesion from 373.....	247	-1,472	N.	Death resulted from intercurrent disease. Very large rabbit.
Bovine S.....	699	Culture.....	118	+ 113	N.	Chloroformed.
	375	Culture.....	69	- 450	G.	
	697	Culture.....	77	- 56	G.	Moderately invasive type.
	567	Lesion from 697.....	50	- 425	G.	Widely generalized case.
	764	Lesion from 567.....	65	- 340	G.	Moderately invasive type.
	835	Lesion from 764.....	74	- 453	G.	Costal and diaphragmatic pleuræ largely covered with small, flat, whitish tubercular tumors.
Hog O.....	846	Lesion from 835.....	72	- 538	G.	Mild type of disease.
	539	Culture.....	57	- 340	G.	Widely diffused case.
	576	Lesion from 539.....	12	- 113	L.	Chloroformed; leg broken.
	577	Lesion from 576.....	48	- 340	G.	Rapidly advancing type.
	633	Lesion from 577.....	42	- 906	G.	Do.
	675	Lesion from 633.....	69	- 652	G.	Costal and diaphragmatic pleuræ studded with small tubercular nodules.
	747	Lesion from 675.....	58	- 30	G.	Costal pleura and pericardium support very numerous pearly growths.
	815	Lesion from 747.....	91	- 624	G.	Costal pleura supports numerous tubercular growths.
Sheep M.....	441	Culture.....	96	- 680	G.	Severe type.
	360	Lesion from 441.....	63	- 283	G.	Do.
	602	Lesion from 360.....	58	- 283	G.	Do.
	676	Lesion from 602.....	47	- 210	G.	Do.
	722	Lesion from 676.....	58	- 226	G.	Shows extensive tubercular infection of the intestinal walls.
	788	Lesion from 722.....	52	- 226	G.	Very general infection.
	838	Lesion from 788.....	91	- 396	G.	Do.

VIRULENCE FOR WHITE MICE.

A number of inoculations of white mice were made with tubercle bacilli from each culture. In most cases the results were entirely negative. In others, while there were no microscopic tubercular lesions, there would be noted a considerable enlargement of the kidneys. Microscopic examination of smears taken from kidneys in this condition usually disclosed the presence of numerous scattered bacilli.

So few of the mice developed tubercular lesions, and those which did develop them had so little relationship one with another, that the presenting of the results in tabulated form seems to be sufficient for the purposes of this investigation. It may be stated, however, that the inoculations were uniformly made subcutaneously at the base of the tail.

Table showing results of inoculations of white mice.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of mouse.	Material used for inoculation.	Size of dose.	Days alive.	Result.	Remarks.
Girl I.....	3	Culture.....	c.c. 0.25	87	N.	Chloroformed.
	8	Culture.....	.15	119	N.	
	22	Culture.....	.10	95	N.	
Boy V.....	42	Culture.....	.10	127	N.	Do.
	9	Culture.....	.15	8	N.	Death due to pneumonia.
	12	Culture.....	.25	78	G.	Axillary glands and kidneys contained tubercle bacilli.
	25	Lesion from 12.....	.10	19	G.	Lungs and kidneys contained tubercle bacilli.
	26	Lesion from 25.....	.10	7	N.	Kidneys contained a few tubercle bacilli.
BB.....	27	Lesion from 25.....	.10	17	N.	
	28	Culture.....	.30	61	L.	
	34	Lesion from 28.....	.10	73	N.	Chloroformed.
	4	Culture.....	.25	196	L.	Chloroformed; lungs filled with tubercular nodules.
	17	Lesion from 4.....	1.00	122	N.	Chloroformed; no tubercle bacilli could be recovered.
	31	Culture.....	1.00	26	L.	Tubercle bacilli were found in the kidneys.
	33	Lesion from 31.....	.20	3	N.	Death due to septicemia.
	37	Lesion from cat 54.....	.20	4	N.	Death caused by intercurrent disease.
	38	do.....	.20	4	N.	Do.
	1	Culture.....		196	L.	Tubercular lesions present in lungs.
Sputum C...	16	Lesion from 1.....	1.00	122	N.	Chloroformed; apparently free from tuberculosis.
	32	Culture.....	1.50	17	N.	Lungs, liver and spleen support many tubercular foci.
	35	Lesion from rabbit 794.....	1.00	16	G.	
	48	Lesion from 35.....	.20	25	N.	Precrural glands, liver, and spleen support tubercular foci.
	43	Lesion from rabbit 858.....	.10	26	G.	
	45	Lesion from 43.....	.10	2	N.	Death due to intercurrent disease.
Case XIV...	10	Culture.....	.15	2	N.	Death caused by pneumonia.
	14	Culture.....	.25	12	G.	Lungs and spleen contain tubercular foci.
	20	Lesion from 14.....	.10	53	N.	Lungs and precrural glands contain caseous foci.
	23	Culture.....	.10	56	N.	
	29	Culture.....	.10	78	G.	
Monkey.....	44	Lung of dog 82.....	.10	15	N.	Spleen and precrural glands tubercular.
	46	Culture.....	.10	11	G.	
Bovine S....	2	Culture.....	.25	196	N.	Chloroformed.
	19	Culture.....	1.00	53	N.	Spleen and kidneys swarming with tubercle bacilli.
	8	Culture.....	.10	53	G.	
Hog O.....	21	Lesion from 8.....	.10	94	N.	Point of inoculation and right kidney positive.
	40	Culture.....	.10	69	N.	
	5	Culture.....	.25	10	G.	

Table showing results of inoculations of white mice—Continued.

Source of bacilli.	No. of mouse.	Material used for inoculation.	Size of dose.	Days alive.	Result.	Remarks.
Hog O.....	15	Culture.....	c. c. 0.25	103	N.	Chloroformed; one small healed lesion in lungs.
	30	Culture.....	.10	41	N.	
	41	Culture.....	.10	127	L.	
Sheep M.....	6	Culture.....	.20	25	L.	Death due to pneumonia; tubercle bacilli recovered from seat of inoculation.
	7	Lesion from 6.....	.20	94	N.	Chloroformed; tubercle bacilli recovered from lungs, spleen, and kidneys.
	13	Culture.....	.20	69	G.	
	24	Lesion from 13.....	.10	67	N.	Tubercular foci located in lungs.
	39	Culture.....	.10	69	L.	
	47	Lesion from 39.....	.10	11	N.	

VIRULENCE FOR CATS.

In the experimental work with cats a change was made in the manner of making inoculations, intrathoracic injections being adopted instead of the subcutaneous method used upon the test animals of other species. A dosage of 1 c. c. was uniformly employed, and the usual gauge of density was followed in the preparation of the material from dog-serum cultures which served for the initial inoculations. The custom was followed, as in all the other experiments, of injecting the first animal of each series with a young culture of bacilli grown on solidified dog serum for one, two, or three generations, while the others of the series received the same sized dose of an emulsion of a tubercular gland or other suitable tissue of each preceding animal. When such lesions were not produced recourse was had to the bacilli in culture for the purpose of injecting in continuation of the series.

RESULTS OF CAT INOCULATIONS.

The results from the inoculation of bacilli of culture Girl I bordered so closely upon failure in each instance that no serial tests could be made. Reference to the table on page 51 will show that none but localized lesions were developed, and that these were inclined to heal without affecting the general health of the cat or causing it to lose in weight.

The next culture, isolated from Boy V, presented a marked contrast in the matter of pathogenicity. The first cat—No. 26—to be inoculated with this culture received bacilli from a second subculture on dog serum after thirty days' growth on this medium. The animal quickly began to give evidences of serious illness. It became drowsy and lost its desire for food. Its coat became rough and staring. A sticky fluid began to gather about the corner of the eyes, and this became more and more profuse until a day or two before death the eyes were completely closed. Respiration finally became labored and death occurred on the twenty-third day. This brief outline will

serve to indicate the acute course which the disease followed in the case of cat No. 26. The autopsy showed the following conditions: Both eyes were closed with an accumulation of purulent exudate of greenish-yellow color which was teeming with tubercle bacilli. At the point of injection there had developed a small subcutaneous abscess filled with creamy pus. The axillary lymph gland on this side showed involvement. The principal lobes of the lungs contained hemorrhagic areas filled with various-sized tuberculous foci. The remaining lobes appeared much contracted, having a rough, serrated appearance caused by the pressure of the large quantity of effusion in the thorax. The mediastinal glands were markedly tuberculous. The entire pleural cavity was lined with a cream-colored, tenacious exudate. No lesions of a tubercular nature were noted within the peritoneal cavity.

The other cats in this series offered no material change in the course or character of the disease until cat No. 55, the tenth of the series, was reached. In this instance the cat showed no deleterious effects from the inoculation, but instead was evidently gaining in weight, denoting a great individual resistance to tuberculosis. It was therefore decided after one hundred and twenty-five days had passed to chloroform the animal. It was now found that the bacilli had been widely disseminated throughout the lungs, the liver, and the spleen, and that these organs were thickly studded with tubercular foci. As the greater number of these areas were miliary in character it seems probable that the secondary invasion was of rather recent occurrence, and it is even possible that the natural resistance of cat No. 55 was so great that she would have ultimately recovered had chloroform not been called into requisition. The remaining cats of the series showed no material deviation in results produced from those displayed by the leading members of the series, and no attenuation of virulence of the tubercle bacilli could be detected to which the slow invasion in case of cat No. 55 could be assigned. Taking a review of the series as a whole, it will be seen that culture Boy V is possessed of a high degree of virulence for cats.

Culture BB was less active than the preceding, but produced tuberculosis of a generalized type in each cat inoculated. The first cat of this series was inoculated with a suspension of pure culture of twenty days' growth, which was the twelfth generation since the isolation of the micro-organism. At the post-mortem examination, held one hundred and fifty-four days later, a large, subcutaneous, firm tumor was found at the point of inoculation. The lungs were thickly sown with small foci, but without inflammatory infiltration. The liver and spleen were seriously affected and were enlarged to fully double the normal size. Cat No. 9, second of the series, showed lesions very similar to the above, but did not survive the inoculation for as long a

period. A gain in weight was shown by cat No. 48, the sixth of the series, but all the others in the test showed a decrease.

Considerable variation was shown in the effects of bacilli of culture Sputum C upon the several cats of the series. At the beginning of the series only localized lesions were produced, but increase of virulence was apparently gained by passage from animal to animal. The material used at the initial inoculation had grown for twenty-five days upon dog serum and was of the fourth transfer. On chloroforming cat No. 6, one hundred and thirty-eight days after the inoculation, the only lesion to be found consisted of a tubercular growth some 4 mm. in diameter located in the anterior lobe of the left lung. From this nodule a number of tubercle bacilli could be demonstrated microscopically, and the substance of this lesion served as the material for the transfer of the culture to the second cat of the series, when similar results were obtained. The third cat of the series was pregnant, and the depletion caused by parturition apparently broke down her natural resistance, as the tubercle bacilli gained access to the organs and glands of the abdominal cavity, where they produced countless virulent foci. The disease seems to have gained an access of virulence from this point, and to have later acquired an inflammatory character which persisted until the ninth cat of the series was reached.

Pure culture of Case XIV, removed from dog serum twenty-four days after second transplantation, served for the initial inoculation. Cat No. 27 received this material and was chloroformed seventy-six days later. The lungs and costal pleura supported a few tubercular growths, but these were of feeble character and did not serve to lessen the vitality of the host. Transferred to the next cat of the series no more extensive lesions were produced, and passage to the third brought negative results. From the slight lesions resulting from the inoculation of cultures of Case XIV into cats it was decided to begin a new series, using an emulsion from dog No. 82, as considerable pathogenicity had been shown by this culture for dogs, and from the lesions developed in dog No. 82 bacilli could be obtained that were active and virulent. The results give evidence of the enhancement of virulence attained by Case XIV by passage through dogs. The remaining cats of the series developed rapidly progressing cases of tuberculosis, and there was marked loss of weight and early death in each instance.

The monkey culture proved innocuous to cats, as the post-mortem examination showed no trace of tubercular lesions.

The animals inoculated with culture Bovine S curiously show a gain in weight with the exception of cat No. 40, second of the series. Although the first and second animals developed extensive lesions of

tuberculosis, there was apparently a later loss of virulence, as the alterations were confined almost exclusively to the region of inoculation, and the last two cats in the series had to be chloroformed for necropsy.

Culture Hog O, which has been found to be bovine in every respect, was extremely virulent for cats from the initial test. Generalized tuberculosis of rapid invasion succeeded the inoculation of cat No. 7 with the second generation of a pure culture 27 days old from dog serum. Although the cat survived but thirty-nine days, this interval sufficed for the spread of the disease throughout the tissues of the lungs and into the peritoneal cavity, where the spleen, the liver, and the gastric lymph glands were attacked. In addition to the infection of these visceral organs, the lymph glands of the neck quite uniformly contained caseous foci, and the axillary glands were much swollen and supported numerous caseous centers. The remaining cats were each inoculated with 1 c. c. of an emulsion prepared from a tubercular gland of the preceding animal, and this was followed by marked loss of weight and an acute, generalized form of the disease in each of the members of the series. The average duration of life with the cats of this test was twenty-four and one-fifth days.

The average length of life with the cats of the series of culture Sheep M was but slightly greater than the above, as it reached but twenty-six and one-half days, while the effect of the injection and the extent of the lesions produced were practically the same. The most noticeable point of difference between these two latter cultures was in their adaptation to artificial growth after passage through cats. Culture Hog O quickly developed a luxuriant growth, while it was only by means of long-continued coaxing that culture Sheep M could be shown to have made even the slightest advance.

SUMMARY OF CAT INOCULATIONS.

A study of the results obtained by the injection of the above animals admits of the following conclusions: Cultures Monkey and Girl I are not virulent for cats. Cultures Hog O, Sheep M, and Boy V are the most pathogenic bacilli of those tested on these animals. Bovine S culture has less virulence for cats than the last-mentioned group of bacilli, while of still lower pathogenesis are cultures Sputum C and Case XIV, in the order named. As will be observed in the accompanying table, it is possible in certain instances to increase the virulence of an organism by passage through a series of animals. Similar treatment in other cases when a different bacillus is used fails to produce this enhancement. An organism possessing a moderate degree of virulence is more capable of having such virulence increased than one possessing less pathogenesis, but when the bacillus already possesses a great degree of virulence, this virulence is not capable of being enhanced to the same extent as with less pathogenic

germs. Moreover, bacilli of such low virulence as is indicated in the monkey culture are not susceptible of being exalted in their pathogenicity. Certain irregular results, as cat No. 55, which lived one hundred and twenty-five days, can only be explained by the supposition that the individual resistance possessed by the animal was developed to a far greater extent than is usually observed in that species.

Table showing results of inoculations of cats.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of cat.	Material used for inoculation.	Days alive.	Change in weight (+gain; -loss).	Result.	Remarks.
				<i>Grams.</i>		
Girl I.....	4	Culture.....	104	- 226	L.	Lungs show a few miliary foci of tuberculosis.
	5	Culture.....	133	+1,075	L.	Chloroformed; four tubercular foci found in lungs.
	8	Culture.....	89	- 340	N.	Death due to impaction.
	21	Culture.....	100	+ 213	N.	Chloroformed.
	37	Culture.....	91	+ 453	L.	Chloroformed; few scattered foci found in lungs. Healed.
Boy V.....	52	Culture.....	81	+ 285	L.	Chloroformed; one healed focus in lungs.
	26	Culture.....	23	- 300	G.	Severe inflammatory type.
	29	Lesion from 26.....	19	- 453	G.	Inflammatory type.
	32	Lesion from 29.....	18	- 235	G.	Generalized tuberculosis, most severe in thoracic cavity.
	33	Lesion from 32.....	13	- 313	G.	Generalized tuberculosis, chiefly effective in thoracic cavity.
	36	Lesion from 33.....	15	- 226	G.	Severe generalized tuberculosis.
	39	Lesion from 36.....	14	- 450	G.	Severe inflammatory infection of all thoracic organs.
	41	Lesion from 39.....	18	- 504	G.	Generalized case, with flocculent tubercular exudate in both body cavities.
	44	Lesion from 41.....	34	- 792	G.	
	51	Lesion from 44.....	37	- 482	G.	
	55	Lesion from 51.....	125	+ 423	G.	Chloroformed; lungs, liver, and spleen studded with miliary foci.
	63	Lesion from 55.....	27	- 538	G.	Disease widely generalized.
	67	Lesion from 63.....	19	-1,104	G.	Death resulted from tubercular pleurisy, exudate swarming with tubercle bacilli.
	69	Lesion from 67.....	19	- 962	G.	Do.
	71	Lesion from 69.....	12	- 340	G.	Severe infection of thoracic organs.
BB.....	74	Lesion from 71.....	16	-1,130	G.	Inflammatory type.
	3	Culture.....	154	- 670	G.	Lesions chiefly limited to thoracic cavity, liver, and spleen.
	9	Lesion from 3.....	66	- 120	G.	Lungs, liver, and spleen affected; also mesenteric, gastric, prepectoral, and mediastinal lymph glands.
	18	Lesion from 9.....	177	- 240	G.	Chloroformed; lesions chiefly in lungs, liver, omentum, and mesenteric glands.
	35	Lesion from 18.....	42	- 790	G.	Lesions located in thoracic cavity and spleen.
Sputum C..	43	Lesion from 35.....	27	- 685	G.	Great accumulation of serous fluid in body cavities; lungs, spleen, pleura, and peritoneum affected.
	48	Lesion from 43.....	33	+ 116	G.	Tuberculosis of lungs, pleura, pericardium, and mediastinal and supraprasternal lymph glands.
	54	Lesion from 48.....	67	- 232	G.	Severe generalized case.
	6	Culture.....	138	+ 453	L.	Chloroformed; single tubercular lesion in left lobe of lung.
	17	Lesion from 6.....	118	- 197	L.	Chloroformed; lesions consist of a few nodules in the lungs.
	34	Lesion from 17.....	75	-1,443	G.	Loss in weight partly due to normal parturition; lungs, pleura, spleen, omentum, mesentery, and thoracic lymph glands involved.
	47	Lesion from 34.....	92	- 10	G.	Lesions widely generalized; marked tubercular pleurisy.
	56	Lesion from 47.....	88	+ 396	G.	Lesions chiefly confined to lungs, mediastinal and costal pleurae, and adjacent lymph glands.

Table showing results of inoculations of cats—Continued.

Source of bacilli.	No. of cat.	Material used for inoculation.	Days alive.	Change in weight (+ gain; - loss).	Re-sult.	Remarks.
Sputum C ..	61	Lesion from 56	25	Grams. - 225	G.	Inflammatory tuberculosis of the tissues just recorded and also of the liver.
	65	Lesion from 61	24	- 115	G.	Lesions widespread throughout the body.
	68	Lesion from 65	27	-1,274	G.	Loss of weight partly due to premature parturition; generalized disease with extensive tubercular pleurisy.
Case XIV ..	72	Lesion from 68	23	- 905	G.	Lungs generally involved; liver and spleen have miliary infection.
	27	Culture	76	+ 396	L.	Chloroformed; lungs and costal pleura show a few tubercular growths.
	38	Lesion from 27	87	+ 905	L.	Chloroformed; few slight nodules in lungs and spleen.
	53	Lesion from 38	59	+ 482	N.	Chloroformed.
	59	Lesion from dog 82 ..	43	- 283	G.	Pleura thickly covered with prominent tubercular growths, also pericardium, lungs, liver, pancreas, spleen, kidneys, and lymph glands.
	60	Lesion from 59	19	- 226	G.	Tubercular involvement of the lungs, pleura, pericardium, spleen, liver, and lymph glands.
	62	Lesion from 60	23	- 396	G.	Inflammatory type of tuberculosis, affecting the lungs, pleura, pericardium, liver, thoracic and mesenteric lymph glands, mesentery, and conjunctiva.
	66	Lesion from 62	25	-1,217	G.	Inflammatory type of tuberculosis as above.
	70	Lesion from 66	19	- 368	G.	Lungs, liver, omentum, thoracic and mesenteric glands affected with tubercular exudate in thoracic cavity.
	73	Lesion from 70	21	- 736	G.	Lungs, liver, spleen, and pleura severely tuberculous; purulent tubercular discharge from left eye.
Monkey	1	Culture	222	- 85	N.	Chloroformed.
Bovine S....	12	Culture	101	+ 906	N.	Do.
	30	Culture	74	+ 312	G.	Axillary glands, parietal pleura, lungs, pericardium, suprasternal and mediastinal glands affected.
	40	Lesion from 30	27	- 975	G.	Inflammatory tuberculosis of axillary, bronchial, and mediastinal glands, lungs, pleura, and pericardium.
	46	Lesion from 40	25	+ 312	L.	Lesions confined to lungs.
	50	Lesion from 46	171	+ 453	L.	Chloroformed; disease localized in left lung and adjoining lymph glands.
	64	Lesion from 50	173	+ 342	L.	Chloroformed; has 1 cicatricial area 1 cm. across and a few scattered tubercular nodules in one lobe of lung.
Hog O	7	Culture	39	- 538	G.	Generalized tuberculosis of rapid invasion.
	14	Lesion from 7	23	- 340	G.	Lungs, liver, spleen, mediastinal and gastric lymph glands tubercular.
	16	Lesion from 14	16	-1,105	G.	Severe inflammatory type, involving lungs, spleen, pleura, pericardium, and thoracic lymph glands.
	19	Lesion from 16	19	- 785	G.	Do.
	22	Lesion from 19	24	- 850	G.	Rapidly progressive type with tubercular pleurisy.
Sheep M	10	Culture	44	- 623	G.	Severe infection of thoracic lymph glands, lungs, pleura, and pericardium.
	15	Lesion from 10	36	- 603	G.	Severe infection of lungs, liver, spleen, and lymph glands generally.
	20	Lesion from 15	18	- 905	G.	Inflammatory tuberculosis of thoracic organs with sero-fibrinous tubercular pleurisy.
	23	Lesion from 20	22	- 340	G.	Inflammatory type, as in preceding case.
	31	Lesion from 23	17		G.	Death due to intercurrent disease; numerous tubercular lesions apparent in thoracic cavity.
	31	Lesion from 31	22	- 680	G.	Case of wide generalization.

VIRULENCE FOR DOGS.

Experiments upon a series of dogs were made with each of the principal varieties of tubercle bacilli whose study is here recorded. The natural resistance of these animals to the action of tubercle bacilli was found to be very great, although far from uniform in different individuals. Their natural powers of resistance were so strong that the greater portion of the inoculations made upon them failed to produce any lasting lesions of tuberculosis. These negative results have been attributed to the ideal housing, surroundings, and care provided for the animals, which probably assisted them in overcoming the effects of the injection, since it is usual for dogs to show a greater susceptibility to tuberculosis than is here indicated. A site was selected for the animals at the Bureau Experiment Station on a dry, partially shaded knoll, and individual cages were built well off the ground and covered with wide-mesh wire screens. Each cage was 5 by 12 feet in size and contained a dog box for protection from the weather. That this continuous life in the open air, with excellent care and plenty of food of a good quality, was instrumental in causing the dogs in many cases to resist tubercular infection was proved by the following experiment:

Two dogs, Nos. 90 and 92, of practically the same size and weight, were inoculated simultaneously under the skin of the shoulders each with 2 c. c. of a suspension of a 58-day old culture from dog No. 88. The former was placed in a warm, poorly ventilated, and improperly lighted room, while the latter was placed in one of the above-mentioned cages in the open air at the Experiment Station. The animals were similarly fed and received equal attention. They were chloroformed forty-six days later, at which time No. 90 had lost four times as much weight as No. 92. The necropsy showed the carcass of the former to have a fistulous tract on both shoulders about 10 mm. in diameter, leading along the subcutaneous tissues for 6 to 8 cm., and surrounded by firm, fibrous tissue and tubercular growths. Both prescapular glands were enlarged and contained three or four caseous foci, about the size of peas. The lungs contained numerous translucent tubercular areas scattered through all the lobes. On the other hand, the autopsy on No. 92 showed a small superficial ulcer at each point of injection, but the prescapular lymph glands, lungs, and all other organs were without lesions. Such a striking contrast is here shown of the effects of environment, or rather of hygienic and unhygienic treatment, that we believe the conclusion is warranted that the dogs in this series of experiments were made more resistant by the character of their surroundings.

Small dogs were used in these tests in all cases. Mongrel fox terriers, beagles, black and tans, or pugs, weighing from 15 to 24

pounds, represent the type of animal selected. These were inoculated by injecting the tuberculous suspension or emulsion immediately beneath the skin on both sides of the neck, just in front of the shoulder, at the beginning of the experiment. Other methods were introduced later, as will be seen by reference to the table on page 57, and some variation in the amount injected will also be seen.

Four dogs died during the course of the test, but in only one instance—that of dog No. 83, culture Boy V—could death be assigned to the tubercular invasion. All others were chloroformed after various periods of time had elapsed.

RESULTS OF DOG INOCULATIONS.

Culture Girl I may be considered nonvirulent for dogs.

Subcutaneous inoculations of culture Boy V produced no serious lesions. By the use of intrathoracic inoculation this culture was brought to a degree of virulence which enabled it to produce generalized tuberculosis in dog No. 83. This animal received 1.4 c. c. of a saline suspension of a thirty day's growth of this culture and died sixty-seven days later. The necropsy showed the lungs to be filled with small, round, glassy foci, and the costal, diaphragmatic, and visceral pleura to support numerous yellowish growths varying in size from 0.5 to 5 mm. in diameter. The kidneys contained diffused necrotic areas in the cortical portion. The right eye was partly closed from conjunctivitis, and the purulent discharge therefrom contained numerous tubercle bacilli. An emulsion prepared from a pleura tubercle of this carcass was injected subcutaneously into dog No. 88, and ninety-two days later the animal was asphyxiated. The post-mortem disclosed two tubercular swellings the size of hickory nuts at the seats of injection. The prescapular glands were enlarged and inflamed. The lungs contained many small foci, some glassy and firm and others soft and inflammatory. The pericardium, mediastinal, and suprasternal lymph glands, liver, spleen, kidneys, omentum, and mesenteric glands all supported numerous foci of tuberculosis.

Culture BB was not sufficiently pathogenic for dogs to cause spread of the disease away from the seat of the inoculation except when used intrathoracically. Even then the lesions were few and well circumscribed. This was likewise true when 2 c. c. of an emulsion of the tubercular pericardium of cat No. 54 was injected subcutaneously.

The first three dogs inoculated with a pure culture of Sputum C failed to show any lesions of tuberculosis when chloroformed from seventy-seven to one hundred and thirty days later. The fourth dog received subcutaneously 2.4 c. c. of an emulsion of the suprasternal lymph gland of cat No. 34. This dog died twenty-one days later from

an internal hemorrhage caused by a ruptured liver. On post-mortem examination the points of injection were slightly inflamed and a few foci of tuberculosis were apparent in the lungs, from which tubercle bacilli were recovered. Two cubic centimeters of an emulsion prepared from these lungs were thereupon injected subcutaneously into dog No. 76, which was chloroformed eighty days later. The only lesions apparent were a very few scattered whitish foci in the lungs, in which no tubercle bacilli could be found. Dog No. 85 received 2 c. c. of an emulsified axillary gland of rabbit No. 858 subcutaneously. After being chloroformed sixty-three days later the necropsy showed a small ulcer at the seat of injection on the left side, beneath which was a slender, soft, tubercular swelling about the size of a silver dollar. No other lesions were apparent.

Culture Case XIV was used upon a series of six dogs, and generalized tuberculosis resulted in each case. The sixth of the series, No. 97, appeared unusually resistant, as the organisms were not discovered in any of the visceral organs. It was found by using an emulsion of tissues from dog No. 82 that increased pathogenicity had been acquired for cats and rabbits by this culture during its retention in the canine organism.

The monkey culture was without pathogenicity for dogs by subcutaneous application.

Culture Bovine S was unable to produce progressive tuberculosis even after passage through a series of cats.

Culture Hog O appeared to have slightly greater virulence for dogs when first isolated than the average culture possesses, but not sufficient to cause regularly the development of tubercular lesions. The first of the series was inoculated with bacilli removed from dog serum five weeks after transplanting. The resulting lesion was a single tubercular abscess the size of a hen's egg. On section, well-defined walls were shown inclosing a mass of tenacious, fairly translucent pus, which much resembled tuberculous sputum. In this material tubercle bacilli were identified by the use of the microscope. Passing some of this material on to the second dog—No. 57—no tubercular change was produced at the point of inoculation. The lungs at autopsy were seen to contain a few scattered, glassy foci, which were apparently not advancing. The kidneys were studded with necrotic foci ranging in size up to that of a pinhead, but contained no tubercle bacilli. No effects followed the passage of tubercle bacilli from these pulmonary lesions on to the third dog—No. 70. The fourth dog—No. 78—died fifty-two days after an intratracheal inoculation with 0.8 c. c. of a seventh subculture of twenty-eight days' growth. The lungs were found after death to support a few scattered glassy foci, which were apparently healed and within which no

tubercle bacilli could be discovered by microscopic examination. The mesenteric glands were greatly enlarged and congested, but no tubercular affection could here be seen. It was evident that the death of the dog was caused by enteritis.

No deleterious effects followed the injection of dogs with bacilli from culture Sheep M, and tubercle bacilli could not be demonstrated in any of the lesions produced.

SUMMARY OF DOG INOCULATIONS.

It is of interest to note that in all these tests there has usually been manifested a marked tendency toward the encapsulation of the tubercular focus whenever the injected organisms succeeded in gaining a temporary foothold immediately after their introduction. Lesions developed in the lungs are usually isolated, small, and glassy. There was no tendency toward the formation of soft, progressive, coalescing masses, but on the contrary, every effort of nature seems to have been exerted in the confinement of the intruding organisms within the smallest possible space and in surrounding them there by an impenetrable wall.

It will be observed from the appended table that the subcutaneous inoculations of 2 c. c. of physiologic saline solution in which tubercle bacilli were suspended in the amount uniformly used gave negative results in most instances. Used in this manner cultures Girl I, BB, Sputum C, and Monkey produced no tubercular alterations. The lesions developed when Boy V, Bovine S, Sheep M, and Hog O cultures were used in the manner employed with the other varieties in the comparative tests were very slight and showed no tendency to progress toward generalized invasion.

Only one of the cultures here described has been found to possess sufficient virulence for dogs to produce positive results in each instance of its application to them. The Case XIV culture has shown peculiar virulence for members of the canine family, and as it has been passed from dog to dog its pathogenesis has been found to be constant for these animals, although its effects upon other animals have shown that it possesses but a comparatively low degree of virulence in general.

The only death produced by tubercle bacilli in this experiment was in the case of dog No. 83, injected intrathoracically with a pure culture of Boy V bacilli. The fact that other bacilli were similarly injected without causing generalized lesions, if we exclude Case XIV, indicates that while the method was more severe than the subcutaneous injections the Boy V bacilli were more virulent than those which failed to produce equal alterations by a similar injection.

Table showing results of inoculations of dogs.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of dog.	Material used for inoculation.	Dose.	Days alive.	Result.	Remarks.
Girl I.....	37	Culture.....	c. c.	179	N.	Intrathoracic inoculation; glassy foci in lungs and kidneys; no tubercle bacilli recovered.
	55	Culture.....	2	121	N.	
	72	Culture.....	1	114	L.	
Boy V.....	81	Culture.....	1.5	93	N.	Free from tuberculosis.
	42	Culture.....	2	122	L.	Tubercular lesions at seat of inoculation, and 4 small glassy foci in lungs.
	54	Lesion from 42.....	2	5	N.	Cause of death, pneumonia.
	62	Culture.....	2	116	L.	Numerous glassy foci from 1 to 5 mm. in lungs; few caseous centers in mesenteric glands; no tubercle bacilli could be found in any organ.
	73	Lesion from cow 302..	2	87	L.	All lesions healed; no tubercle bacilli identified in several foci in apices of lungs.
	83	Culture.....	1.4	67	G.	Intrathoracic inoculation; death due to generalized tuberculosis.
BB.....	88	Lesion from 83.....	1	92	G.	Widespread infection.
	36	Culture.....	2	202	N.	All organs normal.
	56	Culture.....	2	119	N.	Intrathoracic inoculation; lungs contained numerous tubercular foci.
	71	Culture.....	1	112	L.	Intrathoracic inoculation; few glassy foci in lungs apparently healed.
Sputum C...	80	Lesion from 71.....	1.5	95	L.	Two small encapsulated foci in lungs fully healed.
	84	Lesion from cat 54...	2	107	L.	
	40	Culture.....	2	130	N.	Intra-abdominal inoculation.
	53	Culture.....	2	77	N.	Tubercle bacilli found in a few foci in lungs.
Case XIV...	69	Culture.....	2	102	N.	A few healed foci were found in lung.
	76	Lesion from cat 34...	2.4	21	L.	Small tubercular ulcer at seat of inoculation, from which cultures were recovered.
	77	Lesion from 76.....	2	80	L.	Point of inoculation, prescapular glands, lungs, pleura, and liver tuberculous.
	85	Lesion from rabbit 858	2	63	L.	Mediastinal glands, lungs, liver, and kidneys affected.
	63	Culture.....	2	117	G.	Intrathoracic inoculation.
	74	Lesion from 63.....	2.5	83	G.	Points of inoculation, prescapular glands, spleen, lungs, and kidneys affected.
Monkey	82	Lesion from 74.....	1.5	50	G.	Points of inoculation, prescapular glands, spleen, lungs, and kidneys affected.
	86	Lesion from 82.....	2	76	G.	Points of inoculation, prescapular glands, lungs, mediastinal glands, kidneys, and spleen affected.
	93	Lesion from 86.....	2	64	G.	Lesions confined to seats of inoculation, prescapular glands, axillary and precutaneous glands and lungs.
	97	Lesion from 93.....	2	221	G.	
	32	Culture.....	1	249	N.	No trace of inoculation remaining.
	59	Culture.....	3	112	N.	No tubercle bacilli could be identified in the numerous small glassy foci in the lungs.
Bovine S.....	38	Culture.....	2	116	N.	Local lesion; no tubercle bacilli demonstrated in the few retrogressive pulmonary foci.
	64	Culture.....	2	110	L.	Single tubercular abscess in left axillary region.
Hog O.....	75	Lesion from cat 40...	2	89	L.	Lungs contain translucent foci 1 to 2 mm. in diameter.
	43	Culture.....	2	112	L.	Intratracheal inoculation.
	57	Lesion from 43.....	2	115	L.	Intratracheal inoculation; death due to intercurrent disease; lungs have a few small foci, which seem to be healed.
	70	Lesion from 57.....	1	104	N.	No evidence of tuberculosis in any gland or organ.
Sheep M....	78	Culture.....	0.8	52	L.	No tubercle bacilli could be identified in the 4 or 5 glassy foci in the parenchyma of the lungs.
	41	Culture.....	2	144	N.	
	58	Culture.....	3	112	L.	

a These animals died; all others were killed.

VIRULENCE FOR GOATS.

Owing to the difficulties encountered in obtaining goats for experimental purposes, the number of inoculations made upon these animals was quite limited. However, as the goat is so similar in most respects to the sheep, the record of the work with goats will be given as a complement to the tests upon other animals. These animals were all subjected to the tuberculin test before the inoculation of tubercular cultures.

The inoculations were made by injecting physiologic salt solution, in which the usual proportion of bacilli had been suspended, subcutaneously on each side of the neck just in front of the shoulder. The amount used varied. Three of the goats received 1 c. c. on each side, while with cultures Girl I, BB, and Bovine S the amount injected was double that quantity, 2 c. c. being used upon each side.

RESULTS OF GOAT INOCULATIONS.

The material from culture Girl I was the second subculture, of twenty days' growth. Although 4 c. c. were injected, the infection did not extend past the lymph glands adjacent to the points of inoculation. At the post-mortem examination no local lesion was found where the inoculations were made, but the prescapular glands were enlarged and firm and contained numerous degenerated foci filled with material of a caseous nature.

In the test of culture Boy V the usual amount of 2 c. c. was inoculated subcutaneously. The culture used was a four and one-half weeks' growth of the second generation. The great virulence of this culture was attested by the fact that the above inoculation caused the death of the animal in thirty-seven days. When examined post-mortem the carcass was found to be much emaciated. At the point of inoculation on each side was a firm encapsulated mass some 5 cm. in diameter, composed of necrosed and infiltrated connective tissue with hemorrhagic areas throughout. The prescapular glands of either side were moderately enlarged and badly tuberculous. The lymph glands of the axillary region were all more or less involved. The lungs were filled with tubercles varying in size from a pin head to a pea. All lobes were more or less involved.

A young kid some 2 months old received 0.75 c. c. of an emulsion of a prescapular gland of sheep No. 15 (Boy V bacilli) on each side of the neck. Asphyxiated one hundred and six days later, tubercular tumors were found on each side at the points of inoculation, each containing an amount of soft, creamy pus about equal in bulk to a medium-sized bean. The prescapular glands were enlarged and contained numerous scattered, calcified areas. Many foci ranging in size from a pin head to a pea were located in the

lungs. These were all gritty on section from the calcareous deposits, and tubercle bacilli were present only in limited numbers.

Four cubic centimeters of Culture BB were used in the test upon goat No. 5, the material being taken from dog serum after twenty-nine days' growth. The animal was chloroformed at the expiration of two hundred and thirty-two days. No remains of any lesion at the points of inoculation could be found. The prescapular glands were severely affected, many tubercular foci with caseo-calcareous contents being present. The principal lobe of the left lung contained at its anterior border numerous coalescent foci of tuberculosis which on section showed many centers of caseation. The remaining portion of the lobe consisted of three isolated cavities containing semifluid pus. In the most anterior one the degeneration was of a more organized nature. The walls of the vomica presented true abscess-like surfaces with a granulating appearance. These cavities were in direct communication with several bronchi. The caudal lobe was sparsely sprinkled with small, millet-seed foci of apparent recent origin. The posterior half of the principal lobe of the right lung contained one large caseous abscess cavity of the size of a closed fist. The contents consisted of a semifluid caseous material which likewise was in direct communication with bronchi. The walls of this cavity appeared similar to those of the cavity in the left principal lobe. The remaining part of the lobe was thinly sprinkled with small foci of tuberculosis. These small millet-seed foci were likewise scattered over the ventral and the caudal lobes. Scattered throughout the spleen tissue were small, circumscribed tubercular nodules of shot-like appearance, very gritty on section.

Fatal results followed on the ninety-fifth day after the inoculation of a goat with 2 c. c. of a suspension of bacilli from culture Sputum C, twenty-eight days old, and of the second generation. At the post-mortem examination there was noted a thickening of the subcutaneous tissues at the points of inoculation. The tumors thus formed were broad, flat, and about 2 cm. in thickness. The axillary glands were enlarged, but no tubercular foci could be detected. The lungs contained two tubercular areas, one in each principal lobe the size of a man's fist, made up of isolated and confluent tubercles from 2 to 5 mm. in diameter. Many of these foci were badly broken down, their centers showing on section a flocculent degenerative deposit. Cover-glass preparations showed under the microscope large numbers of tubercle bacilli of the human type.

The injection of 2 c. c. salt solution suspension of tubercle bacilli from the monkey culture 4½ weeks old and of the first generation gave negative results. On chloroforming the animal two hundred and forty-eight days later no trace of the inoculation could be found at the autopsy.

Culture Bovine S was used in the amount of 4 c. c., second generation, of twenty-six days' growth. The animal died of generalized tuberculosis on the fifty-fifth day. The prescapular glands were found greatly enlarged and supporting numerous caseous areas. The axillary glands were similarly affected. A chain of small lymphatic glands extending along the side of the trachea showed numerous caseous foci. The left submaxillary gland also contained a tuberculous focus about 0.5 mm. in diameter. The suprasternal, bronchial, and mediastinal glands presented small tubercular areas. The lungs were quite congested, and scattered thickly throughout the whole parenchyma and over the various surfaces were caseous nodules from 3 to 5 mm. in diameter. The parietal pleura supported four or five areas of miliary tubercular growths. The portal lymph gland contained one minute focus, but no further tubercular change within the abdominal cavity was noted.

SUMMARY OF GOAT INOCULATIONS.

In summing up the results obtained by the use of these six different cultures upon goats it will be observed that the Monkey and Girl I cultures continue to be the least pathogenic of the series, while Boy V and Bovine S cultures are the most virulent. Cultures Sputum C and BB stand as of intermediary pathogenicity, the former causing the death of a goat in ninety-five days, while the latter did not kill, but produced such an extensive involvement of the lungs that it was remarkable that the animal could continue to live with such a limited amount of lung tissue remaining unaffected. The resistance of young animals to tuberculosis is clearly shown by the inoculation of the 2-months-old kid No. 9 with an emulsion of the Boy V bacilli recovered from sheep No. 15.

Table showing results of inoculations of goats.

[G denotes generalized tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of goat.	Dose.	Days alive.	Result.	Remarks.
Girl I (culture)	7	c. c.	210	L.	Chloroformed; no lesions except in prescapular glands.
Boy V (culture)	3	2	37	G.	Animal died showing extensive tuberculous lesions of progressive type.
Boy V (lesion from sheep 15).	9	1.5	106	G.	Young kid. Chloroformed; points of inoculation, prescapular glands, and lungs tuberculous.
BB (culture)	5	4	232	G.	Chloroformed; disease extended to near-by glands and spleen and caused vomice in lungs.
Sputum C (culture)	6	2	95	G.	Animal died showing tuberculosis of pulmonary type.
Monkey (culture) ..	4	2	248	N.	Chloroformed.
Bovine S (culture).	8	4	55	G.	Animal died severely infected with tuberculosis.

VIRULENCE FOR SHEEP.

The material used in the experiments upon sheep was prepared and inoculated in a manner similar to that employed in the tests upon goats.

RESULTS OF SHEEP INOCULATIONS.

Culture Girl I failed to produce any lesions of tuberculosis in the sheep to which it was applied.

Culture Boy V was of sufficient virulence to produce generalized lesions in each sheep of the series. The first received bacilli of twenty-one days' growth upon dog serum, fourth generation, and showed on post-mortem examination prescapular glands that were swollen and very slightly tubercular. The lungs were closely studded with miliary tubercles, while the costal pleura of the right side of the animal showed scattered foci upon its surface. The mediastinal glands were slightly affected, and a single caseous focus was located in one of the mesenteric glands. In the second of the series—No. 104—inoculated with an emulsified lymph gland of the above animal, a more severe type of disease was developed. Tubercular tumors formed at the points of inoculation, and tubercular infection extended to the prescapular glands, where it manifested itself by the formation of several small abscesses, in which tubercle bacilli were scantily present. All lymph glands of the thoracic cavity were enlarged and supported tubercular foci. The lungs were sprinkled with tubercular foci of various sizes up to 10 mm. in diameter. (Plate IV.) In the liver a few caseous nodules were formed. A lymph gland situated at the lesser curvature of the stomach was greatly enlarged through the growth of tubercular formations. Calcareous tumors the size of a turkey's egg formed at each point of inoculation in the next sheep of the series—No. 118—which received an emulsion of a lymph gland of sheep No. 104. The prescapular glands contained numerous calcified foci. The lungs were very slightly affected with firm, glassy foci. In the mesenteric glands numerous caseous foci were developed.

Culture BB produced no extension of tuberculosis past the prescapular glands of the first sheep of the test. In the second the lungs and liver were slightly involved in addition to the lesions in the prescapular glands, while in the third of the series a more severe infection of lungs and thoracic glands followed, and the lesions in the prescapular glands contained a large proportion of calcified material.

In the test of culture Sputum C light infection of lungs and prescapular glands was at first obtained, but attempts to pass the infection to the next of the series were unsuccessful. For the third sheep of this test an emulsion of an axillary gland of rabbit No. 858 was used, with the result that a widespread infection followed. At the points of inoculation firm calcareous tumors formed to which the skin was closely attached. In the subcutaneous tissues along the inferior surface of the neck were scattered numerous spherical tubercular growths the size of millet seeds. The infection of the

prescapular glands was indicated by calcified foci, each of which was surrounded by a zone of inflammation. The lungs were studded with foci ranging from 1 to 6 mm. in diameter. The bronchial and mediastinal glands were swollen and contained many areas, some caseous, others of a calcareous nature. In the abdominal cavity tubercular lesions were found in the liver, in the portal lymph gland, and in various mesenteric glands.

The first sheep inoculated with tubercle bacilli from culture Case XIV died on the tenth day from another cause. Sheep No. 101, which was thereupon inoculated with bacilli of the second generation, grown upon dog serum for twenty-seven days, developed a few cheesy foci in the submaxillary glands and a large number of them in the prescapular glands. The lungs contained numerous glassy foci and one undergoing degeneration. The intervertebral thoracic glands showed tubercular infection. The liver was affected throughout with foci varying in size from a point to a bean. The second sheep of the series—No. 62—inoculated with an emulsified lymph gland of the above animal, showed enlarged prescapular glands with roughened surfaces. The left lung was adherent to the costal walls over a circular area some 25 mm. in diameter, and in the various lobes of the lungs small foci were scantily sprinkled. Of these about one half appeared glassy and healed, while the other half were inflamed and progressive. A few softened foci were in the mediastinal glands, and there was a slight infection of liver and kidneys. An emulsion of the lung of dog No. 82 was tested upon sheep No. 114 with widespread infection as the result. The submaxillary, sublingual, prepectoral, prescapular, precrural, mediastinal, portal, and gastric lymph glands all contained caseo-calcareous foci, while many of the mesenteric glands were swollen and inflamed. The lungs were sown throughout with tubercles of all sizes up to beans, the spleen was also permeated with calcareous nodules, and the liver and kidneys gave evidence of slight infection. Scattered about at random over the mesentery were various small tubercular bodies which were firm and hard when pressed between the thumb and finger. On microscopic examination tubercle bacilli were found in limited numbers.

Judging from the extension of the lesions, and the apparent degree of virulence manifested by culture Bovine S, it may very properly be classed with Case XIV as to its virulence for sheep. The first sheep of the series, receiving 2 c. c. of a suspension of bacilli from dog serum thirty-two days old and of the second generation, developed tubercular abscesses at each point of inoculation, miliary tuberculosis of the lungs, and a slight infection of the axillary glands. Double the quantity was injected into the next sheep of the series, and a greater infection was thereby obtained. At each point of

inoculation the skin was adherent to the underlying tissues over an area some 50 mm. broad. Under each of these areas was an abscess some 20 mm. across, which was full of thick, cheesy pus. The adjacent lymph glands were filled with caseous foci. The lungs contained numerous glassy foci. The diaphragmatic pleura supported many fibrous neoformations. The bronchial and mediastinal lymph glands were studded thickly with small tubercular foci. Slight infection of liver and spleen was noted. Four cubic centimeters of an emulsion of the prescapular gland of sheep No. 13 was injected into the third of the series—No. 16. A few abscesses containing cheesy pus in which a few tubercle bacilli were demonstrated formed in the prescapular glands, in the lungs, and at the points of inoculation. The infection was of a mild type. Following this a sheep—No. 117—was inoculated with 2 c. c. of an emulsion from an axillary gland of rabbit No. 764. The prescapular and axillary glands developed caseo-calcareous foci. The bronchial and mediastinal glands were enlarged and contained many calcareous foci. The lungs supported a few glassy foci. The liver contained about 12 small yellowish-white lesions, while the portal and mesenteric lymph glands were thickly sown with small caseous lesions.

SUMMARY OF SHEEP INOCULATIONS.

None of the sheep inoculated with the various cultures tested died from the inoculation. With the exception of sheep No. 99, all of the animals were prepared for the investigation of the progress of the disease by asphyxiation with either chloroform or illuminating gas. For this reason it becomes difficult to determine the relative virulence of these various bacilli for sheep, as it is necessary to conclude from the extent and character of the lesions produced rather than from the length of time that the animal survived the injection. With this in view it will be possible to class the culture Girl I as without virulence for sheep, and cultures Bovine S, Case XIV, and Boy V as the most pathogenic for these animals. Culture BB appeared to be of lower virulence than the latter, while Sputum C was slightly less pathogenic than culture BB.

It is also interesting to note in the experiments conducted with sheep and goats that the latter appeared to be much more susceptible to the invasion of tubercle bacilli than sheep. This fact seems to be well borne out by an examination of the abattoir statistics of various countries, which indicates that sheep are also more resistant to natural infection with tuberculosis than goats. This point does not seem to have been given much prominence heretofore, but should receive greater consideration in the future in speaking of the relative susceptibility of these two species of animals.

Table showing results of inoculations of sheep.

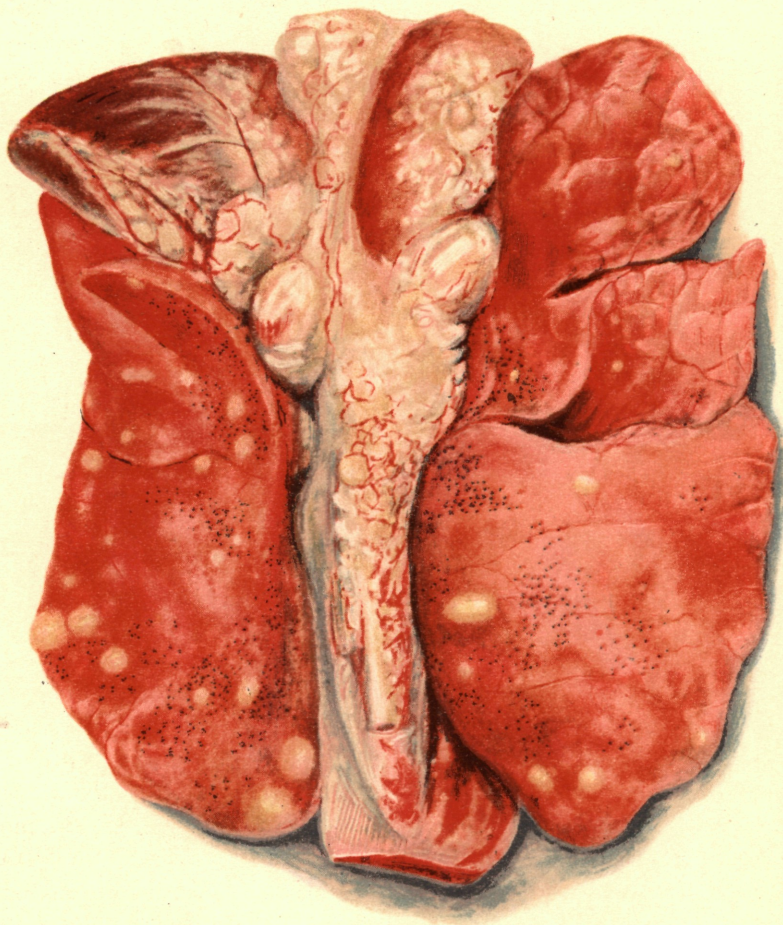
[G denotes generalized or advancing tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of sheep.	Material used for inoculation.	Dose.	Days alive.	Result.	Remarks.
Girl I.....	63	Culture.....	c. c. 2	122	N.	No trace of tuberculosis found.
Boy V.....	15	Culture.....	2	44	G.	Prescapular glands, lungs, costal pleura, and mesenteric gland affected.
	104	Lesion from 15.....	2	94	G.	Thoracic glands, lungs, liver, and mesenteric glands affected.
	118	Lesion from 104.....	2	106	G.	Prescapular glands, lungs, and mesenteric glands contain tubercular foci.
BB.....	18	Lesion from goat 5....	2	166	L.	Prescapular glands alone tuberculous.
	103	Lesion from cat 43....	2	83	G.	Prescapular glands, lungs, and liver tuberculous.
	116	Lesion from 103.....	2	107	G.	Prescapular glands, lungs, and thoracic glands affected.
Sputum C..	94	Lesion from goat 6....	2	152	G.	Prescapular glands and lungs slightly affected.
	2	Lesion from 94.....	2	132	N.	No evidence of tuberculosis could be found.
	119	Lesion from rabbit 858	2	120	G.	Many lymph glands, lungs, and liver thickly sown with tubercular foci.
Case XIV...	99	Culture.....	4	10	?	Died of intercurrent disease.
	101	Culture.....	2	101	G.	Submaxillary, prescapular, axillary, and thoracic glands, lungs, and liver tuberculous.
	62	Lesion from 101.....	4	89	G.	Lungs, mediastinal glands liver, and kidneys affected.
	114	Lesion from dog 82....	2	119	G.	Numerous lymph glands, lungs, liver, spleen, kidneys, and mesentery tuberculous.
Bovine S...	98	Culture.....	2	60	G.	Lungs, axillary glands, and points of inoculation involved.
	13	Lesion from 98.....	4	75	G.	Prescapular glands, lungs, pleura, liver, and spleen tuberculous.
	16	Lesion from 13.....	4	93	G.	Prescapular glands and lungs tubercular.
	117	Lesion from rabbit 764	2	102	G.	Prescapular, axillary, and mesenteric glands, lungs, and liver tuberculous.

VIRULENCE FOR CATTLE.

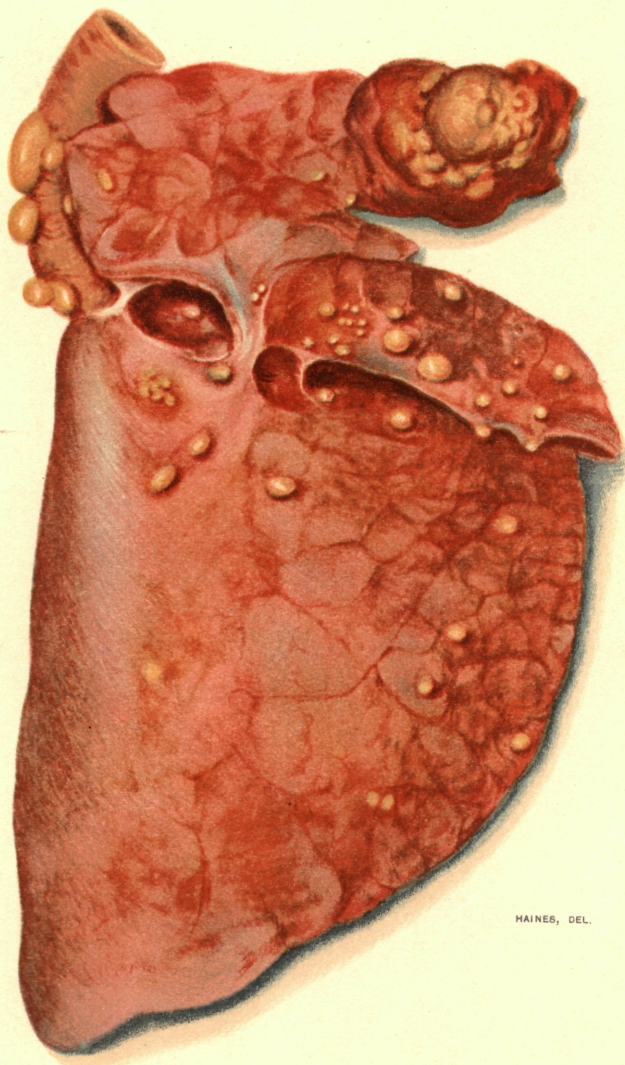
Greater interest attended the effects of the various cultures upon cattle than upon any other species of test animals, and the number of instances in which generalized invasion followed subcutaneous inoculation goes far to establish the fact that human tuberculosis is transmissible to cattle. No test was made with the cultures represented by culture 19, monkey, and those represented by culture Girl I proved incapable of causing any form of tubercular lesion. No test upon cattle was made with the three cultures derived from hogs, nor with the three bovine cultures represented by culture Hog O, neither with the culture Sheep S, because of the manifestly bovine character of them all.

Ravenel had already proved his BB culture infective for cattle, and we can safely add our culture Boy V to the list of cultures derived from human sources that have previously been shown by other investigators to possess pathogenic powers for cattle.

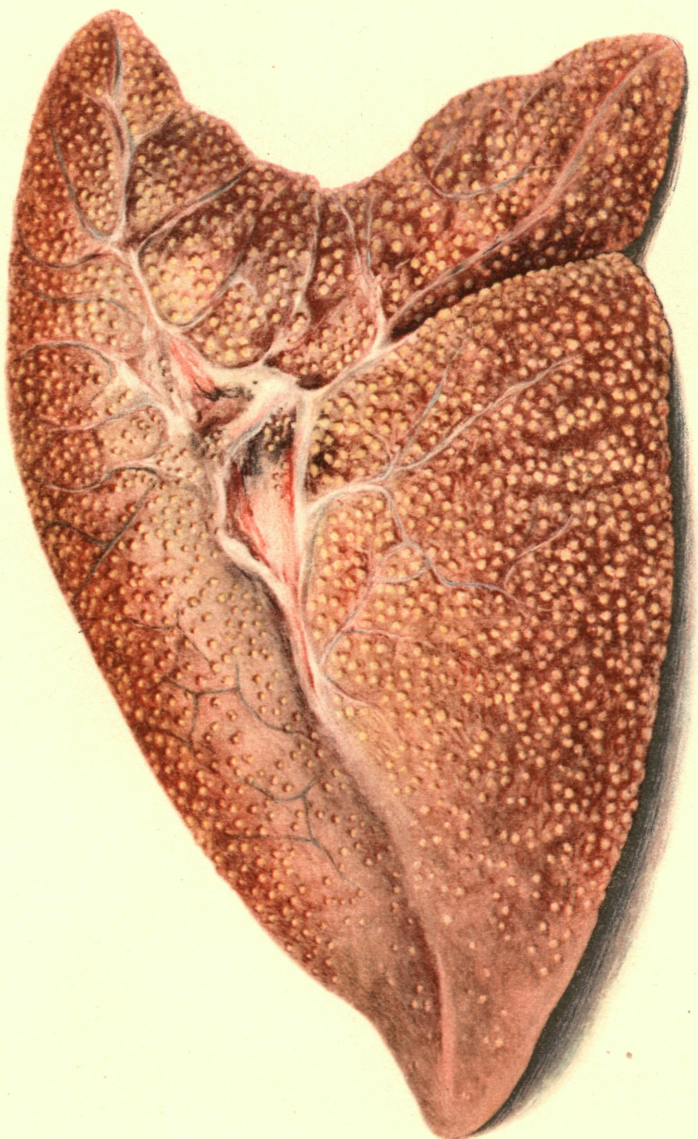


HAINES, DEL.

LUNG OF SHEEP NO. 104, INOCULATED SUBCUTANEOUSLY
WITH HUMAN TUBERCULOSIS (BOY V).



LUNG OF HEIFER NO. 302, INOCULATED
SUBCUTANEOUSLY WITH HUMAN TUBERCULOSIS (BOY V).



HAINES, DEL.

MILIARY TUBERCULOSIS
AFFECTING LUNG OF HEIFER NO. 297, INOCULATED SUBCUTANEOUSLY WITH HUMAN
TUBERCULOSIS (B. B.).

Of the 20 cattle submitted to the test, 3 failed to show any resulting tubercular lesion; 2 gave an extension of the micro-organisms no further than to the prescapular or to the prescapular and retro-pharyngeal glands; the remaining 15 developed tuberculosis of a generalized character. Out of these 15 animals, 3 died from the infection, and another was killed when in a moribund condition. It is of further interest to note that all of these fatalities were brought about by the use of infectious material derived from human sources, 2 direct from the culture and 2 from tissue emulsion after passage through cats.

The cattle inoculated were from 10 to 18 months of age, except a portion of those used in testing BB culture, for which purpose two animals were used that had reached an age of 20 and 22 months, respectively, and one—No. 318—that was 4 months of age. No young calves were used in any case. The tuberculin test was applied to each of the cattle, without reaction, previous to the experiment.

All inoculations were made subcutaneously on each side of the neck just in front of the shoulder, and 2 c. c. was uniformly introduced at each seat of injection except in the case of No. 340, Case XIV, where an accident necessitated the use of a smaller amount—1.25 c. c. on each side.

RESULTS OF CATTLE INOCULATIONS.

As has already been stated, culture Girl I failed to cause any form of tubercular lesion; neither was there any material rise in temperature resulting. The fourth generation of a thirty days' growth of dog-serum culture was used in the inoculation.

Culture Boy V, at the other extreme of virulence, caused tuberculosis of the severest type. The fourth generation of twenty-four days' growth upon dog serum was used for the initial inoculation, being applied to heifer No. 302. On the following day there was an elevation of temperature aggregating 2.8° F., and for about two weeks there was considerable fluctuation of temperature. After this the normal level of body temperature was gradually approached, but there was an evident disturbance of the animal's health. By the time five weeks had elapsed firm tumors had developed at each point of inoculation, the prescapular glands were plainly affected, and the general appearance of the animal indicated increasing unthriftiness. It was chloroformed on the one hundred and twenty-seventh day following the inoculation, and at the necropsy firm, irregular tubercular tumors 15 to 25 cm. in diameter were found at the points of injection. The adjoining lymph glands were swollen to the size of duck's eggs and supported several areas of hemorrhagic inflammation, also numerous foci of caseo-calcareous degeneration,

while the retropharyngeal and submaxillary glands were slightly enlarged and inflamed, but contained no tubercles or bacilli.

A representation of the lesions of the lungs is given in Plate V. Many of the nodules on the visceral pleura projected from 1 to 8 mm. beyond the surface of the organ, and their similarity to the nodules frequently seen in "grape disease" of cattle, following natural infection, was very striking. The parenchyma supported numerous caseous areas, as indicated by the conglomerate, irregular nodular mass presented in the caudal lobe of the illustration. The numerous growths appearing upon the pleura were also suggestive of "grape disease." On the parietal pleura of the right side seven or eight tubercular nodules with constricted bases were located. The early stages of the disease were also apparent, as broad areas of hemorrhagic fibrous neoplastic growth were to be seen. On the left side similar fibrous neoformations were present, but the yellowish-gray projecting nodules were lacking. The diaphragmatic pleura likewise showed the early stages of this affection. The pericardium was also covered with small grapelike clusters and a hemorrhagic fringe of a tuberculous character. Numerous grapelike nodules appeared upon the serous covering of the thymus glands, especially upon the left side. Bronchial and mediastinal—especially the posterior—lymph glands were enlarged, edematous, and contained many calcified foci together with petechial hemorrhages. The liver supported some 20 tubercular nodules varying from 3 to 20 mm. in diameter. Five situated near the surface were especially worthy of note because of the fact that they projected some 6 mm. above the surface of the liver and were attached to it by constricted or pedunculate bases. Upon the peritoneal surface of the spleen a few irregular areas of fibrinous growth were present, with three tuberculous nodules attached to the splenic capsule. On section four additional foci were discovered in the parenchyma of the organ. An edematous condition was noticeable in the sublumbar and portal lymph glands, but no tubercle bacilli were demonstrated. No more complete invasion of the system by tubercle bacilli could have been expected had bovine bacilli been used.

The clinical history of the above animal applies equally well to the two following members of the series—Nos. 313 and 345. The former received 2 c. c. of an emulsion of the prescapular gland from No. 302 in front of each shoulder, while No. 345 was injected in the same manner with a similar quantity of an emulsion obtained from the prescapular gland of No. 313. There were no abrupt alterations of temperature, but in each instance a gradual increase of some three degrees was noted, followed by a season of fluctuation and afterwards by a slow return of normal conditions. The result of the post-mortem

examination of No. 302 may with equal propriety be taken to represent the character of the post-mortem tubercular lesions shown by Nos. 313 and 345. Extensive invasion of the tissues by the organisms injected was disclosed in each instance, and a tendency toward the formation of grapelike bodies upon the pleura, pericardium, and upon the outer surfaces of the lungs and liver was also uniformly manifested. Prescapular, bronchial, and mediastinal glands were involved in both cases, as was the parenchyma of the liver and lungs. No. 345 also showed numerous fibrinous growths on the capsule of the spleen.

That the virulence of Boy V culture has not been decreased by continuous growth upon artificial media for thirty-one generations over a period of three years is indicated by the result obtained with two young cattle by the subcutaneous injection of a 32-day-old culture of the above generation of this organism. On June 21 bull calf No. 458 was injected as previously described. On August 3 the reaction at the seat of injection had become so extensive that large, flat swellings were observed, and both prescapular glands had increased to the size of duck's eggs. The general condition of the animal was poor and its appearance dull and languid. On August 8 the calf was so weak that it could scarcely walk. It was found dead on the morning of August 15, fifty-five days after the injection. On autopsy, lemon-sized tumors were observed at the seat of inoculation, which on section were found to consist of a uniform mass of tuberculous tissue. The connective tissue surrounding the tumors was edematous and sprinkled with minute caseous tubercles from 1 to 3 mm. in diameter. The submaxillary, post-pharyngeal, and tracheal lymph glands were intensely congested and enlarged, and contained tuberculous lesions. The prepectoral, prescapular, bronchial, and mediastinal lymph glands also appeared greatly enlarged and entirely tuberculous. The lung was uniformly sprinkled with innumerable necrotic tuberculous masses from 1 to 5 mm. in diameter. The distance between these different masses was about 5 mm. The liver contained one tuberculous focus about 3 mm. in diameter on the convex surface. The portal lymph glands presented two or three minute tuberculous nodules. The intestines, mesenteric glands, spleen, and other organs and glands were normal, with the exception that the lymph glands generally were more or less edematous.

Heifer calf No. 462 was inoculated with material similar to that used on the preceding calf, and on the same date. By July 30 the swellings at the seat of injection had gradually increased until they were 5 by 8 by 10 cm. in size, and the prescapular glands were each about the size of apples. The appetite of the animal was capricious, its temperature fluctuating, and its general condition poor. The ani-

mal was found dead on August 29, sixty-nine days after the injection. On autopsy, two large tumors were observed at the seats of injection, about 8 by 10 by 10 cm. in size; both of these swellings, on section, were found to be composed almost entirely of tuberculous masses from one-half to 2 cm. in diameter. The prescapular glands were greatly enlarged and completely tuberculous. The lung was evenly sprinkled with innumerable tuberculous foci, from 2 to 10 mm. in diameter. The bronchial, mediastinal, prepectoral, and suprasternal lymph glands appeared very much swollen, and contained numerous caseous areas. The post-pharyngeal lymph glands were also enlarged and tuberculous. The spleen contained a few small foci of tuberculosis. The liver showed no disease, but the hepatic lymph glands were enlarged, edematous, and contained each from one to six small tuberculous areas. The precrural lymph glands appeared slightly enlarged and revealed a few small tuberculous masses. The superficial inguinal glands were greatly swollen, but contained no tuberculous lesions. This was in the strictest sense a case of completely generalized tuberculosis.

The bacilli used in the initial test of culture BB upon cattle consisted of a twenty-six days' growth on dog serum and had had a total growth under artificial conditions of about sixteen months. The fourteenth generation was the only one available, and although the culture evinced considerable pathogenicity for the bovine race when used in pure culture, it is possible that its continuous artificial cultivation had lessened this virulence somewhat. This conclusion seems justifiable when the results here obtained are compared with those gained by Ravenel a year previously. At each point of inoculation (in heifer No. 266) a firm tumor gradually developed, which ruptured spontaneously about three months after the inoculations were made. These continued to discharge more or less freely up to the time of slaughtering the animal. The material cast off had the appearance of thick, creamy pus, in which many fragments of caseous débris were mixed. Post-mortem examination one hundred and seventy-eight days after the injection disclosed open ulcers at each point of inoculation. The prescapular glands were badly tuberculous. On sectioning the swollen glands they were found to be filled with thick, creamy pus and broken-down glandular tissue. The retropharyngeal lymph glands showed several tubercular foci, and in the surrounding tissues many yellowish tubercular areas were noted. There was no extension to either the thoracic or the abdominal cavity.

A second heifer, No. 258, was at once inoculated with an emulsion of one of the prescapular glands of No. 266. The resulting lesions were very similar to those found in No. 266 in their extent, but instead of the moist, creamy pus which filled each tubercular area, dense calcareous deposits were present. There was, moreover, an

extension into the peritoneal cavity, where a scattering of small tubercular growths was found attached to the capsule of the spleen.

In order to test the effect of rapid passage through cats upon the BB tubercle bacilli, the third heifer—No. 297—was inoculated with an emulsion of omentum from cat No. 43, the fifth cat in the series. The resulting disease was marked by its rapid onslaught and wide extension. There was an elevation of temperature of 2° within a few days, and then a period of comparative regularity at points varying between 102.8° and 104° F. After this, up to the time of the death of the animal, the temperature was regularly high, flesh and strength were lost rapidly, and a persistent cough was present. At the post-mortem examination firm tumors with caseo-calcareous contents were noted at the seats of inoculation. The prescapular glands were of the size of a man's fist and contained many areas of tuberculous degeneration. On viewing the thoracic cavity, the visceral and parietal pleuræ were found to be firmly adherent. In like manner, where the various lobes of the lung came in contact with each other, the adjacent surfaces of the visceral pleura had become almost completely fused by a fibrous connective tissue growth, which could only be separated by the application of force. Scattered here and there over the external surface of the pericardium were small masses of nodules, each mass containing from 5 to 12, while a chain of similar nodules extended over the entire length of the organ from base to apex along the right side. The bronchial and mediastinal glands appeared badly affected throughout. The lungs were nearly filled with tubercular nodules 2 or 3 mm. across. These nodules were more numerous in the anterior portion of the lung, where the tubercular tissue was more plentiful than the normal lung substance. (See Plate VI.) The organisms had also invaded the abdominal cavity, and the parenchyma of both liver and kidneys were thickly sown with nodules varying from 1 to 5 mm. in size. A few similar nodules were present in the spleen, and the mesenteric glands also showed wholesale invasion.

Carried to the second animal—No. 318—of this series the resulting disease was less acute, but was still of a very active type and caused pronounced elevation of temperature with rapid emaciation of the animal. The post-mortem examination held ninety-seven days after injection showed walnut-sized areas of tuberculosis at the seats of inoculation, with marked involvement of the adjacent prescapular lymph glands and dissemination to the thoracic cavity, where the mediastinal glands and lung tissue presented numerous small caseous foci of disease. The third animal—No. 342—injected with an emulsion of the prescapular gland from the above calf produced lesions very similar to those found in the first of the series, but they were of slower development, and were not accompanied by emaciation or

by marked increase of body temperature. When chloroformed one hundred and twenty-five days later, soft caseous tumors the size of pigeon's eggs were present at the points of inoculation. The prescapular glands were irregularly enlarged and calcareous. The costal pleura was very largely covered with inflamed fibrous patches composed of coalescing tubercular growths. The diaphragmatic pleura and the pericardium showed similar growths. The lungs contained numerous superficial fibrous growths, and the lung tissue throughout was permeated by countless small inflammatory foci, varying in size from a pin point up to a millet seed. The bronchial and mediastinal glands were enlarged and studded with tubercular foci the size of millet seeds and mostly calcified. On the external surface of the rumen was an area 13 by 20 cm., over which round, smooth nodules, in size up to a pea, were scattered. The omentum and the peritoneum showed wide areas roughened by coalescing tubercular growths and fibrous neoformations. The liver supported numerous small glassy foci. The spleen showed inflammatory tubercular growths in size up to beans on both surfaces of the organ.

Culture Sputum C failed to cause any tubercular lesion when a pure culture twenty-seven days old and of the fourth generation was injected. When accentuated by passing through a series of rabbits, generalized tuberculosis followed its introduction beneath the skin of cattle. The first animal—No. 312—thus treated evinced an acceleration of respiration within fifteen days after the inoculation, and at the same time a temperature of 106° F. The animal became unthrifty, but did not at any time show great emaciation. It was chloroformed on the one hundred and fifty-ninth day. At the autopsy the points of inoculation were found to support tumors, some 20 mm. across, each with a center filled with thick, yellowish pus. The retropharyngeal, submaxillary, and prepectoral glands were all enlarged and edematous and contained numerous small centers of tuberculosis. The prescapular glands were seriously involved, containing many large foci of yellowish calcareous matter. The bronchial and mediastinal glands were gritty on section and were thickly studded with tubercular foci. The parietal pleura supported upon its costal and diaphragmatic surfaces many inflammatory fibrous tubercular growths. The lungs contained 12 or more scattered grayish tubercular foci, located both deeply and superficially. The omentum presented an area some 50 mm. broad, extending across its entire width, to which a fringe of fibrous tubercular growths was attached. The liver contained both superficial and deep tubercular lesions, and its portal lymph glands were enlarged, containing foci of tuberculosis.

Carried to the second of the series by the injection of an emulsion of the prescapular gland, similar conditions were caused in regard

to temperature elevation, general course of the disease, and extent of tubercular lesions developed.

This animal—No. 341—was killed on the one hundred and twenty-second day, after having shown a previous high fever and a general loss of condition. At the necropsy each point of inoculation showed a tumor about 50 by 100 by 40 mm. in size. Both tumors were composed of masses of tubercular nodules some 2 mm. in diameter, hard and calcareous. The prescapular glands were about 65 by 50 by 100 mm. in size, firm and gritty. Their surrounding membranes supported numerous scattered nodules in size from millet seeds to small beans. In places the adjoining muscle tissue had been invaded by tubercle bacilli and numerous scattered foci among the muscle fibers had resulted. The lungs showed superficial growths upon all lobes, in size up to beans. By sectioning it was observed that the nodules were scattered all through the lung substance and most of them were calcified and encapsulated. The mediastinal glands appeared swollen, and contained numerous calcified tubercular nodules. The costal pleura showed areas which supported pearl-like growths, some of them being pedunculated; in places they were collected together in groups comprising 10 to 12 grapelike bodies. The pleural nodules varied in size from millet seeds to marrow beans, and numerous fibrous neoformations were also present. The spleen showed slight infection. The kidneys each contained a few scattered foci. The liver had several small foci, as did the portal lymph glands.

The inoculation of yearling No. 304 with bacilli of culture Case XIV, taken from dog serum, caused no marked elevation of temperature or perceptible impairment of health. At the post-mortem examination, held one hundred and twenty-five days after the inoculation, each point of inoculation was found to support an abscess some 60 mm. across, which was filled with creamy pus loaded with short, straight tubercle bacilli. The prescapular glands were double their normal size, edematous, and had been invaded by tubercle bacilli from the adjoining local lesion. The inoculation of No. 314 with material from the local lesion of No. 304 gave only negative results.

An emulsion from the lung of rabbit No. 884, injected with Case XIV bacilli, was next inoculated beneath the skin of yearling No. 340. Instead of the customary dose of 4 c. c. given to the other cattle, this one received but 2.5 c. c., owing to the overturning of the tube in which the emulsion had been placed.

The temperature rose from 101.6° to 106° F. within fifteen days, then gradually subsided almost to normal, where it remained without material change until the animal was killed. The general appearance of the animal was affected; it was not as bright as usual, and its appetite was only fairly good at the time of acme of the fever. Chlo-

reformed on the one hundred and fourth day, fluctuating tumors some 50 mm. across were found at the seats of the injections, from which creamy matter bearing many tubercle bacilli was liberated on section. The prescapular glands were greatly distended through the formation of calcareous areas of a great variety of sizes. The mediastinal glands were swollen and were sown with scattered calcareous nodules the size of millet seeds.

An emulsion of Case XIV bacilli obtained from the lung of cat No. 60 was next used, and this material caused the death of yearling No. 343 in thirty-five days. The disease assumed the most acute form from the very outset. The temperature rose to 107° F. on the sixteenth day, and at no time afterwards receded below 106° F. until just previous to death. Throughout the course of the disease the appetite was impaired, the respiration affected, and the animal became dull and weak. At the post-mortem examination the picture of acute inflammatory tuberculosis was displayed. The two points of inoculation consisted of firm tumors, 40 by 75 by 100 mm., composed of newly formed fibrous tissue, permeated with small caseous foci. The lymphatic glands of the neck and throat were invaded by the infection. The axillary and prescapular glands were similarly infected. The costal pleuræ were roughened by many fibrous tubercular neoformations. The pericardium supported numerous tubercular growths in size up to millet seeds. The lungs were distended and filled by myriads of small tubercular foci, each surrounded by zones of inflammation. The bronchial and mediastinal glands were greatly enlarged, the latter about four times their normal size, and were filled with caseous foci and inflammatory areas. The liver, the spleen, and the kidneys were studded with miliary foci, and the omentum had sprinkled over its visceral surface many fibrous neoformations interspersed with small, round tubercular growths, in size ranging up to millet seeds. The internal inguinal glands were inflamed and showed several tubercular areas.

Some months after the inoculation of No. 343 a further test was made by inoculating No. 344 with a culture from dog No. 93. This was done as a means of comparing the virulence of tubercle bacilli of Case XIV after passage through a series of cats, exemplified by yearling No. 343, with the same culture after passage through a series of dogs, and later recovered in pure growth upon dog serum. Considerable difficulty was experienced in getting a growth started from the tubercular lesions of dog No. 93, and the material finally used upon heifer No. 344 was taken from a feeble growth upon dog serum.

At the post-mortem examination of No. 344, held one hundred and sixty days after the inoculation, all mediastinal glands were found to be affected throughout with caseo-calcareous tubercular centers, from

which tubercle bacilli were readily recovered in pure culture. The lungs also supported small, yellowish, gritty growths scattered throughout both the deep and the superficial tissues of the various lobes. From these lesions pure cultures were also readily recovered, direct transfer to glycerin-agar even resulting in quite a vigorous multiplication.

In testing culture Bovine S, inoculations of the usual amounts were made in the manner adopted in all previous cattle experiments, using bacilli of the third generation and of thirty-six days' growth upon dog serum. A transitory elevation of temperature followed in a few days. Firm tumors developed at each point of inoculation, but the prescapular glands at no time appeared to be enlarged. It was found at the post-mortem examination one hundred and sixty days later—the animal having been chloroformed—that the seat of injection on the right side had developed a small elongated subcutaneous abscess, which was filled with soft, creamy pus. On the left side was a cystic tumor some 50 by 50 by 100 mm., filled with watery pus. The right prescapular gland contained a single circumscribed tubercular abscess some 16 mm. across, filled with yellowish, cheesy pus. In the opposite gland three abscesses with similar contents had formed. These lesions were about 12 mm. in diameter. The mediastinal glands were slightly increased in volume and contained a few scattered caseous foci about the size of millet seeds. No other lesions of tuberculosis could be found in the carcass.

SUMMARY OF CATTLE INOCULATIONS.

The results obtained by the subcutaneous injection of these various tubercle bacilli into cattle indicate very conclusively that man may harbor tubercle bacilli of such pathogenicity as to cause the death of the cattle into which they are inoculated. The question of the apparent exalted virulence of several of these organisms after passage through a series of animals will not be discussed at present, but will be treated in another publication.

The negative effects following the injection of the culture of Girl I bacilli are in perfect accord with the results of all the other previously described experiments with these organisms. Culture Boy V again shows its apparent intimate relationship to the various bovine organisms, and in fact is much more pathogenic than the Bovine S culture. That the Boy V organism has retained this high degree of virulence over three years is another indication of the fixity of its character, and can not be accredited to the coincidence of extreme susceptibility of the two animals upon which it was last tested.

Cultures Sputum C and Case XIV were undoubtedly of such a low degree of virulence originally as to prevent their infecting cattle,

but by manipulation it was found possible to cause a spreading tuberculosis in the animals inoculated.

Similar injections of the BB bacillus confirmed the results obtained by Ravenel in his intravenous and intraperitoneal inoculations of cattle with this organism, which placed it among the group of bacilli of the bovine type derived from children. The effect of the inoculation of Bovine S culture showed it to be less virulent for cattle than is usual with bovine cultures. Indeed, it may be considered an aberrant bovine organism, which leads to the inference that there is a certain range of pathogenesis possessed by bovine bacilli which is similar to, even if more limited than, the variation of virulence that we know exists among bacilli obtained from man.

Table showing results of inoculations of cattle.

[G denotes generalized or advancing tuberculosis; L, local lesions; N, negative.]

Source of bacilli.	No. of animal.	Material used for inoculation.	Age of animal.	Days alive.	Result.	Remarks.
			<i>Mos.</i>			
Girl I.....	299	Culture.....	12	153	N.	Chloroformed; no lesions of any kind.
Boy V.....	302	Culture.....	12	127	G.	Chloroformed; widely generalized tuberculosis.
	313	Lesion from 302.....	18	169	G.	Do.
	345	Lesion from 313.....	13	108	G.	Do.
	458	Culture.....	10	55	G.	Died; widely generalized tuberculosis.
BB.....	462	Culture.....	10	69	G.	Do.
	266	Culture.....	12	178	L.	Chloroformed; light infection; points of inoculation and prescapular and retropharyngeal glands alone involved.
	258	Lesion from 266.....	20	198	G.	Chloroformed; points of inoculation, prescapular glands, and splenic capsule affected.
	297	Lesion from cat 43.....	22	58	G.	Moribund; widespread tubercular infection.
	318	Lesion from 297.....	4	97	G.	Chloroformed; points of inoculation, prescapular and mediastinal glands, and lungs involved.
	342	Lesion from 318.....	16	125	G.	Chloroformed; widespread tubercular infection.
Sputum C..	300	Culture.....	12	158	N.	Chloroformed; no tubercular lesions found.
	312	Lesion from rabbit 741.....	16	159	G.	Chloroformed; widely generalized tuberculosis.
Case XIV...	341	Lesion from 312.....	15	122	G.	Do.
	304	Culture.....	12	125	L.	Chloroformed; necrotic areas at seat of injection with slight extension to prescapular glands.
	314	Lesion from 304.....	15	119	N.	No lesions of disease found.
	340	Lesion from rabbit 884.....	12	104	G.	Chloroformed; points of inoculation and prescapular and mediastinal glands severely attacked.
	343	Lesion from cat 60.....	12	35	G.	Died; generalized invasion of visceral organs.
	344	Lesion from dog 93.....	14	160	G.	Chloroformed; involvement of lungs and mediastinal glands.
Bovine S...	316	Culture.....	12	160	G.	Chloroformed; points of inoculation and prescapular and mediastinal glands involved.

HISTOLOGIC EXAMINATION OF LESIONS PRODUCED.

In the microscopic examination of the tubercular lesions observed in the various experiment animals used in this investigation, specimens of the lungs, the liver, and the lymph glands, and also of an occasional

spleen and kidney, were examined in order to ascertain if any differences could be detected in the alterations produced as a result of the various kinds of tubercle bacilli used in these experiments. Numerous sections were made from the tissues of the animals in the various series, and in most cases all of the above-mentioned organs were obtained when diseased for the purpose of a comparative histologic study. In the case of dogs the tissues examined were chiefly the lungs, as the disease failed to spread to other organs in many cases. In cattle, goats, and sheep the lesions of the lungs, livers, and lymph glands were the ones principally studied, while in cats and rabbits all of the first-mentioned tissues were examined. It will be almost impossible to give any satisfactory description of the lesions observed in mice, as the effect of injection on these animals was irregular and lacked uniformity.

In all cases the tissues were embedded in paraffin after having been hardened and fixed in Zenker's fluid or in increasing strengths of alcohol. Several methods of staining were adopted, including alum-hematoxylin and carbol-fuchsin, hematoxylin and eosin, and Weigert's fibrin stain; but where the tubercle bacilli were to be brought out for comparative study the sections were always stained in carbol-fuchsin, decolorized in 20 per cent sulphuric acid, and counterstained in alkaline methylene blue, as previously stated. This examination revealed a varied picture, depending not only upon the character of the organism injected but upon the age of the lesions and the susceptibility of the animal to the material inoculated.

A determination of such differences as existed was readily made with bacilli from certain sources, but in a number of cases the extent of the lesions, the number of tubercle bacilli present, the amount of caseation observed, or the formation of fibrous tissue was so similar as a rule that it was impossible to note any uniform variations. For instance, it might be stated that in the majority of sections made from the lungs and livers of the series of animals inoculated with the bacilli from culture Sheep M, the largest sized tubercles, the greatest caseation, and the most numerous tubercle bacilli were observed. On the other hand, in those tissues obtained from the Monkey and Girl I cultures the other extreme was quite noticeable. In the lymphatic glands of these various animals tubercle bacilli were more numerous than in the viscera, and it was more difficult to notice differences in these tissues. In attempting to classify the lesions in groups the difficulty is at once met of having all gradations of the disease present in some cases or excessive alterations apparent in a number of animals of a series, but probably only slight lesions in a more resistant animal in this same series. However, such exceptions only prove the rule and will not be taken into consideration in arranging the cultures in groups.

The lesions of animals inoculated with cultures Hog O and Boy V were only secondary to those caused by the sheep bacillus in extent, in the number of tubercle bacilli, and in the activity of the cells surrounding the areas. The next gradation in the quality and quantity of tubercles produced includes, in the order of their severity, BB, Bovine S, Sputum C, and Case XIV. In fact, it might be stated that in this group the BB and Bovine S germs produced lesions slightly in excess and more active, with a greater number of bacilli present, than did the Sputum C or Case XIV, although in many cases—especially in the lungs and livers of rabbits and cats—a great similarity was noted in the lesions produced by all these bacilli, while in the kidneys and spleens the first two of this third group presented more advanced lesions and contained a more generous supply of bacteria.

In describing the lesions observed in the animals receiving cultures Monkey and Girl I, the livers and lungs of cats and rabbits are principally referred to, inasmuch as these organisms failed to infect mice, cattle, dogs, or, to any extent, sheep. The sectioning of necrotic areas in the above tissues shows numerous foci varying in size and due to cell infiltration, including all three of the constituents described as being typical of the tubercle—epithelioid, lymphoid, and, occasionally, giant cells. The larger tubercles consist mainly of epithelioid cells, while lymphoid cells occur at the edges in fair numbers. At the margins of these large areas there is occasionally seen an attempt on the part of the capillaries to enter in order to organize the structure. Caseation may or may not be observed in the central portion of the tubercle, and if present it does not progress to any marked degree. Tubercle bacilli in scant numbers are observed in these tubercles, either free, lying in the caseous matter, or at times within the giant cells or an epithelioid cell. Occasionally several polymorphonuclear leucocytes are seen in varying stages of granular degeneration in the caseated center. In some cases this area of caseation has failed to reveal a single organism after prolonged search, while in other instances bacilli are seen in the process of disintegration, as indicated by the light pinkish tinge of staining.

Directly in contrast to the slight destruction of tissue, the scarcity of tubercle bacilli, and the lack of activity of the cells surrounding the above tubercles are the more acute lesions induced by the bacilli of culture Sheep M. In the organs of animals inoculated with this bacillus the tubercles were large, with well-marked caseated centers, a tendency to progress, and with myriads of tubercle bacilli in the non-caseous as well as in the caseous matter. In fact, in many cases tubercle bacilli and epithelioid cells could be seen in the lymph spaces and occasionally in blood vessels in the apparently normal surrounding tissue.

Giant cells may or may not be found, but there appears to be a

greater number observed in lesions produced by the bacilli of lower virulence than by this culture. These tubercles are probably the most extensive, most acute, and richest in tubercle bacilli of any. The tissues used in this comparison were the lungs, livers, spleens, and kidneys of cats, rabbits, sheep, goats, and mice.

The histologic examination of the lesions produced by cultures Hog O and Boy V were so similar in character that they will be discussed together. These acute, progressive tubercles partake largely of the nature of lymphoid cell infiltration with rapid coagulation necrosis of the central portion. Numerous tubercle bacilli are observed in the caseated centers, from which they may become transplanted on adjoining tissue through the agency of phagocytes, or by the lymphatic vessels. In fact, in these lesions many tubercle bacilli were observed in the lymph spaces of the apparently normal tissue surrounding the tubercle, where they may cause the development of other tubercles. That this has already occurred in certain cases was manifested by the appearance of an occasional single large tuberculous area composed of several similar tubercles. These tubercles also increase in size by peripheral growth as well as by this formation of daughter tubercles in the adjacent tissues. In the extent of the tubercles produced and in the amount of caseation present it was very difficult to differentiate these lesions from those produced by the sheep culture, but regarding the quantity of bacilli present it might be stated that while the amount was excessive in the lesions of both these cases there did not appear to be the myriads of bacilli that were noted in the sheep-culture lesions.

In the alterations produced by cultures BB, Bovine S, Sputum C, and Case XIV there were many tissues in which they all seemed to have about the same extent of alterations, while in others, especially noted in the kidneys and lungs of certain cats and rabbits, it was observed that the BB and Bovine S cultures produced a somewhat more extensive tubercle with greater caseation and a larger number of tubercle bacilli present than did Sputum C and Case XIV, but not to such an extent as to be included in the group occupied by cultures Hog O and Boy V.

It was thought inadvisable to compare the microscopic appearance of the lesions produced in dogs by Case XIV culture with those caused by the other organisms. For some inexplicable reason culture Case XIV had a peculiarly active virulence for dogs, producing lesions markedly in contrast to the other cultures of this group, or, in fact, even to those cultures (Sheep, Boy, and Hog) more pathogenic for other animals. In all tubercles in dogs, other than those produced by Case XIV, the tendency toward repair seems to have been stronger than the destructive agency, as indicated by the gradual transformation of the epithelioid cells into connective tissue ele-

ments. The small, glassy, translucent tubercles were completely circumscribed by the formation of connective tissue, which was also seen to have replaced in certain cases the central caseated area, causing the tubercle to be transformed by the connective and fibrous tissue cell infiltration.

It must be understood that in placing these various cultures in the above groups they were classified after taking into consideration the most advanced tubercles that could be detected in the various tissues. Of course it was frequently possible, even in the lesions caused by the sheep culture, to observe metastatic tubercles of cell infiltration of such recent origin that the amount of caseation, the number of bacilli, and the size of the tubercle would be far inferior to the well-developed and more aged tubercle produced by a less virulent culture, such as the Girl I bacillus.

CHEMICAL REACTION OF GLYCERIN-BOUILLON CULTURES.

In 1905 Theobald Smith³² published the description of a simple method for distinguishing the bovine from the human type of tubercle bacilli in glycerin-bouillon cultures. Following closely the instructions there given, a number of the cultures already discussed in this article were made accustomed to growth upon fluid media and then tested as to their chemical reaction after growth in flasks upon glycerin-bouillon under the prescribed conditions.

Although marked differences are apparent in the adaptability of various cultures to growth upon these media, the chief interest is centered upon the effect of their continued cultivation upon the reaction of glycerin-bouillon. And from these effects, as stated by the author of the article just cited, "mammalian tubercle bacilli may be divided into two groups, one corresponding to the human, the other to the bovine type."

Cultures which adhere closely to the human type in their virulence for test animals and in their morphology will as a rule accommodate themselves to their new environment when transferred to fluid media with greater readiness than cultures of the bovine type, and the more luxuriant growth which is evinced by them when grown upon solidified serum is also displayed in the thick, wrinkled covering which they form upon the surface of the fluid. Cultures whose members belong strictly to the bovine type are slow to show growth, and when at last the surface of the fluid is completely covered the layer formed by their growth is delicate and thin for some weeks and does not approach in profuseness the culture of the human type, except in cultures that have been for a long time subjected to artificial cultivation. The acidity of the glycerin-bouillon used in these tests varied before inoculation from 1.85 to 2.1 per cent acid to phenolphthalein.

The most satisfactory results from this method of testing will be obtained from cultures that have been cultivated for several generations upon artificial media and that have become thereby so modified that they grow readily when transplanted from tube to tube.

In the article above referred to we read:

When flasks of glycerin-bouillon in layers $1\frac{1}{2}$ to $2\frac{1}{2}$ cubic millimeters deep are inoculated with scales of tubercle bacilli from glycerin-agar cultures the floating masses soon begin to expand, and after one or two months a complete membrane will have formed over the relatively clear bouillon. The bacillar masses which fall to the bottom at the outset increase but slightly in size. This mode of surface growth of tubercle bacilli was shared by all mammalian races I have examined after they had acquired a certain saprophytic habit, and it is well known to all those who cultivate tubercle bacilli for the preparation of tuberculin. If ordinary bouillon prepared from fresh beef with 3 to 5 per cent glycerin added be used, and if the acidity be made equivalent to about 2 per cent of normal acid, phenolphthalein being the indicator, the reaction of the bouillon during the formation of the membrane will take one of two directions. It may gradually approach the neutral point and reach an acidity or an alkalinity of about 0.1 to 0.2 per cent when the membrane is complete and remains so, or else the acidity may diminish during the first month, to increase again during the second and fluctuate more or less if the observation be continued, but it does not reach the neutral point. * * *

The reaction curve of the bovine cultures moves steadily from the acid to the feebly alkaline level, where it remains. The latter is reached in some lots of bouillon as early as three weeks, in others somewhat later. Very rarely it does not reach it. The reaction curve of the human cultures is more complicated. During the formation of the membrane the acidity is gradually reduced and may fall as low as 0.3 per cent. After the membrane is completed and as it grows thicker and enlarges in all directions by pushing the margin up the sides of the flask or even down into the fluid, the acidity gradually rises again, and it may reach 2 per cent. More commonly it fluctuates between 0.5 and 1.5 per cent. In no instance have I seen any of these cultures become neutral or faintly alkaline to phenolphthalein.

A great variety of cultures have been tested by this method and a large number of flask growths have been carried through a sufficiently long period of time to give the resulting reactions shown in the accompanying tabular form. While we do not look upon this method as capable of determining with any degree of accuracy the origin of any particular culture, it will be noticed that Boy V in both instances approached closely to the neutral point, and that the four flasks of Hog O culture similarly reduced the acidity of the glycerin-bouillon quite materially. It may therefore be concluded that both these organisms are of bovine type, although one was isolated from the mesenteric gland of a child and the other from the mesenteric gland of a hog. Several of the flasks from Girl I culture also showed considerable reduction in acidity, but others of the same culture failed to react in this manner, and these cultures, together with the Sputum C and Case XIV growths, support the conclusions reached by Smith.

Table showing chemical reactions of tubercle bacilli in glycerin-bouillon. (Cultures used in present experiments.)

Culture.	Variety.	Date of inoculation.	Time required to cover surface.	Reactions on stated days after growth covered surface (+alkaline; -acid).									
				Days.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	P. ct.
Girl I.....	Rabbit 1250...	May 5, 1905	56	10	-1.3	24	-1.1	43	-2.0	68	-3.5		
Do.....	Rabbit 1220...	Feb. 9, 1906	49	10	-0.9	24	-0.4	41	-0.4	63	-1.0		
Do.....	do.....	Mar. 12, 1906	23	10	-1.1	24	-0.6	41	-1.1	63	-1.1		
Do.....	Cow 361.....	Jan. 2, 1906	36	14	-0.9	24	-0.8	36	-0.6				
Do.....	do.....	Apr. 12, 1906	20	15	-1.3	37	-1.1	51	-0.8				
Do.....	do.....	do.....	19	14	-1.2	36	-0.7	50	-0.4				
Boy V.....	Dog 88.....	Jan. 6, 1905	55	12	-0.5	29	-0.25	49	-0.3	67	-0.35		
Do.....	do.....	Nov. 3, 1905	49	10	-1.6	30	-1.0	51	-0.6	61	-0.2		
Sputum C.....	Cow 341.....	Mar. 12, 1906	44	10	-3.0	20	-3.3	52	-3.2				
Case XIV.....	Cow 344.....	do.....	32	15	-3.2	32	-3.0	54	-2.7				
Hog O.....	Cat 7.....	July 3, 1905	49	15	-1.4	26	-0.8	40	-0.3	61	-0.2		
Do.....	do.....	Dec. 6, 1905	37	15	-0.9	26	-0.4	36	-0.1	61	-0.1		
Do.....	do.....	Apr. 5, 1906	64	10	-1.2	21	-0.7	30	-0.2	61	-0.4		
Do.....	Cat 22.....	Jan. 30, 1905	45	12	-1.8	21	-1.0	30	-0.3				

In addition to the cultures in the above table, which are those already described in this paper, a number of glycerin-bouillon flasks were inoculated with tubercle bacilli derived from various sources in order to ascertain their chemical effect on this particular medium. The list includes Möller's grass bacillus and bacilli obtained from various wild animals, a dog, a fowl, and four children.

Table showing chemical reactions of tubercle bacilli in glycerin-bouillon. (Cultures derived from various sources.)

Culture.	Variety.	Date of inoculation.	Time required to cover surface.	Reactions on stated days after growth covered surface (+alkaline; -acid).									
				Days.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	P. ct.
Avian.....	Old stock.....	May 12, 1905	26	10	-1.0	25	-0.8	55	-1.5	61	-1.7		
Do.....	Rabbit 1101.....	Jan. 30, 1905	24	10	-1.3	23	-0.9	50	-0.9	65	-0.6		
Do.....	do.....	May 12, 1905	27	10	-1.7	25	-1.6	55	-1.6	65	-1.8		
Grass bacillus.	Möller's.....	do.....	10	10	-1.7	19	-1.6	40	-1.9	68	-1.1		
Bear.....	Old stock.....	do.....	3	14	-1.5	28	-0.8	49	-0.5	70	-0.0		
Do.....	do.....	Nov. 20, 1905	3	14	-2.0	28	-1.0	49	-0.6	70	-0.1		
Baboon.....	Menelik.....	May 12, 1905	31	10	-2.0	21	-1.1	35	-1.3				
Monkey.....	Bedford.....	Nov. 7, 1906	43	10	-1.1	19	-1.2	40	-1.2				
Do.....	do.....	Apr. 10, 1906	35	14	-0.8	30	-1.2	42	-1.0				
Bovine.....	III.....	Dec. 6, 1905	22	10	-2.0	15	-1.0	30	-0.8	40	-1.0		
Do.....	do.....	Dec. 22, 1905	10	11	-1.4	20	-0.7	30	-0.4	40	-0.4		
Do.....	do.....	Mar. 12, 1906	10	10	-1.6	19	-1.2	32	-0.8				
Canine.....	Stock.....	May 5, 1905	57	12	-1.8	30	-1.2	40	-2.0	55	-3.1		
Deer.....	New Mexican.....	July 7, 1905	24	10	-2.3	28	-1.0	43	-3.0	54	-3.2		
Do.....	do.....	Dec. 19, 1905	18	21	-1.3	30	-0.9	45	-0.6	53	-0.5		
Do.....	European.....	Feb. 8, 1906	33	18	-1.2	30	-1.1	50	-0.9				
Do.....	do.....	Mar. 28, 1906	34	18	-1.2	30	-0.9	50	-0.9				
Do.....	Sambur.....	Feb. 1, 1906	49	10	-1.2	20	-1.2	35	-2.7				
Coati.....	Coati.....	do.....	38	10	-1.0	20	-0.8	33	-0.06				
Do.....	do.....	Apr. 9, 1906	22	16	-1.9	24	-1.6	40	-1.0				
Do.....	do.....	do.....	19	17	-1.6	39	-1.3						
Nilgau.....	Nilgau.....	Mar. 12, 1906	21	10	-1.3	28	-0.8	43	-0.8				
Do.....	do.....	Apr. 9, 1906	10	17	-1.4	30	-0.7	53	-0.6				
Wallaby.....	Wallaby.....	Feb. 1, 1906	37	14	-1.2	22	-0.7	40	-0.4				
Do.....	do.....	Mar. 29, 1906	42	14	-1.2	22	-0.7	40	-0.4				
Do.....	do.....	do.....	38	10	-1.4	24	-0.7	31	-0.4				
Human.....	Case 30, child.....	Dec. 14, 1905	58	10	-1.7	20	-1.2	30	-1.9				
Do.....	do.....	do.....	24	10	-1.7	20	-1.7	30	-2.2				
Do.....	do.....	do.....	44	11	-1.5	21	-1.5	34	-1.8				
Do.....	do.....	do.....	44	13	-1.3	23	-0.9	33	-1.1				

Table showing chemical reactions of tubercle bacilli in glycerin-bouillon—Continued.

Culture.	Variety.	Date of inoculation.	Time re-quired to cover surface.	Reactions on stated days after growth covered surface (+alkaline; -acid).									
				Days.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	Days.	P. ct.	
Human	Case 30, child.	Mar. 10, 1906	16	10	-1.5	20	-1.2	30	-1.2				
Do.	Case 31, child.	Dec. 20, 1905	38	14	-0.8	24	-0.9	34	-0.9				
Do.	do.	Jan. 12, 1906	77	10	-1.5	20	-1.3	30	-2.0				
Do.	do.	Feb. 10, 1906	39	10	-1.4	20	-1.3	30	-2.0				
Do.	do.	Mar. 10, 1906	16	10	-2.0	21	-2.0	30	-2.0				
Do.	do.	Mar. 29, 1906	38	10	-1.6	20	-1.0	34	-1.7				
Do.	Case 32, child.	Mar. 26, 1906	19	14	-0.9	29	-1.7	51	-2.4				
Do.	do.	Apr. 10, 1906	10	12	-2.2	27	-2.8	45	-3.3				
Do.	Case 33, child.	Jan. 19, 1906	32	10	-1.0	14	-0.4	24	+0.2	37	+0.15		
Do.	do.	Apr. 5, 1906	38	10	-1.3	14	-0.6	24	+0.0	37	+0.1		

Referring to the above table, which gives the reaction curves in cultures from miscellaneous sources, it becomes evident that uniformity of results should not be expected. None of the bovine cultures reached the neutral point. Bovine III is an old culture that has acquired during nine years' cultivation considerable saprophytism, but that this should not affect its reaction is shown by the reaction given by the bear culture, which is still older and more saprophytic. It was confidently expected that some of the tests of deer, coati, nilgau, or wallaby cultures would show the development of pronounced alkalinity, as all other tests had shown them to possess many characteristics of the bovine type; but this did not occur, and human culture case 33 was the only one to prove distinctly alkaline and to react in a manner typical of the bovine bacillus.^a

It will thus be seen that this test, while in a number of instances it shows an intimate relation between virulent tubercle bacilli and an alkaline reaction of the glycerin-bouillon, will not give a uniform reaction in a group of flasks which have been prepared and inoculated in the same manner. Nevertheless there is an approach to uniformity, and it is a striking fact that all the cultures derived from the human reacted in a way confirmatory of Smith's results, with the exception of Boy V and case 33 cultures (derived from mesenteric lymph nodes of children), whose difference in reaction curves is explicable on the ground of their bovine type. On the other hand, Bovine III culture for some unaccountable reason failed to give the bovine reaction of these last two organisms or of the Hog O bovine bacillus. The expectation that the wild-animal cultures would give

^a Since these chemical tests have been made it has been found by animal inoculations and biologic tests that cases 30, 31, and 32 are similar to our culture Girl I, but that case 33 is as much bovine in type as our Boy V bacillus, if not more so.

bovine reactions was not realized, and may be explained by the fact that, coming from intermediary hosts, certain modifications of their biochemic characters have occurred, or that, having their own particular hosts, reactions peculiar to these hosts have been obtained, placing them in a group outside of either the human or the bovine class. Furthermore, these results would indicate that such chemical reactions are quantitative, not qualitative, and in cases where aberrant bacilli are studied irregular reaction curves may be expected.

CONCLUSIONS.

As a result of the foregoing experiments performed in this and other laboratories, we believe it justifiable to draw the following conclusions:

1. While certain peculiarities of growth, morphology, and pathogenesis are observed with a fair degree of constancy in bacilli of human origin, nevertheless these characteristics are not universal, and notable exceptions are observed which would confuse those who would attempt to establish their origin by means of such characteristics.

2. A similar degree of constancy in the morphological, biological, and pathogenic characters of the bovine bacillus is generally noted, but a certain range of differences has been observed, which, though apparently more limited than for the human bacillus, is nevertheless suggestive of aberrant forms.

3. Therefore the assertion, based solely on these facts, that a bacillus has a certain origin can only be tentative, as bacilli from man have been found which conform in all respects to bacilli obtained from cattle.

4. Tubercle bacilli of widely different virulence may be encountered in different cases of bovine as well as of human tuberculosis.

5. There is a certain proportion of cases of human tuberculosis in which may be found tubercle bacilli which are pathogenic for cattle.

6. Dependent upon the medium in which grown—whether in vitro or in vivo—tubercle bacilli will present special characteristics in accordance with their different environment, and these characteristics may lead to a supposition relative to their origin, although they are not always fixed or unchangeable.

7. The numerous instances above recorded in which the inoculation of cattle with tubercle bacilli from human sources has resulted in the production of tuberculosis must prove decisively that tubercle bacilli of human origin have been pathogenic to the bovine, or, on the other hand, the bacilli must have been bovine in origin, and in spite of this fact were readily able to obtain a footing within the

human tissues, in either case demonstrating the transmissibility of the differing types from one species of mammals to another.

8. The more the subject is studied the more numerous are the instances noted in which the bacilli appear naturally in animals that are far removed from the species supposed to be their usual host, thus proving that they are not closely limited in their choice of victims and that there is no character possessed by any of these types of tubercle bacilli in one host that may not be found in another host under favoring conditions.

9. Upon examination of the results obtained from inoculating bacilli marked "Boy V" in this experiment it will be at once noticed that it possesses a remarkable degree of virulence, in this respect surpassing many that we have obtained from bovine sources. Culturally, morphologically, and pathogenically this culture conforms closely to the bovine type. Could it be shown to have originated, previous to its attack upon the boy, from a bovine source, its fatal effects upon the child's system would offer incontestable proof of the susceptibility of the human organism to the inroads of the bovine tubercle bacillus. If, on the other hand, the bacilli for many generations past have successively grown upon human hosts only, we must at once admit that in this instance the type approaches so closely to the bovine form that all of the prominent characteristic differences between them have been removed, and we have before us a tubercle bacillus of human origin that has become so modified by peculiar environment that it is no longer a representative except in name.

10. There are human types of bacilli that are similar in morphology and biology, but vary as to their virulence; and, conversely, there are bovine bacilli typical as to form and growth, but less pathogenic than those usually observed.

11. If we exclude culture BB, which has already been included in Ravenel's description of bovine organisms obtained from human tissues, it will be observed that of four cultures obtained from children, one gave all the known reactions to the various tests applied for placing it in the bovine class.^a It will also be noted that of nine cultures of bacilli obtained from sputa, one was entirely beyond the virulence possessed by the others, although not so pathogenic as some organisms coming from bovine animals, but nevertheless indicating the difference in degree of virulence among sputa bacilli. Of the eight germs obtained from cattle, hogs, and sheep, one was

^a Cultures of tubercle bacilli from the mesenteric lymph nodes of five children affected with infantile tuberculosis have been studied since the above work was performed, with the result that one was found of the bovine type, thus making two out of the nine cases of tuberculous children studied in this laboratory that were affected with bovine tubercle bacilli.

observed which had a modified virulence when compared with the high pathogenicity of the others tested, again illustrating the variability of virulence of bovine germs.

12. The question of the transmissibility of tubercle bacilli of bovine origin to man will not be here further discussed from these experiments; but sufficient evidence, in our judgment, has been adduced to warrant the adoption and enforcement of sanitary measures against the use of the meat and the milk of tuberculous animals, and to make it advisable to eliminate all tuberculous cattle from the herd or to sterilize all the milk therefrom. While the greatest amount of tuberculosis in man is undoubtedly caused by its spread from human to human, the frequency of his infection from animals should not be underestimated.

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